STUDIES ON THE BIOSYNTHESIS OF DERMATAN SULFATE

A. MALMSTRÖM and L.-Å. FRANSSON
Department of Physiological Chemistry 2, Chemical Centre
University of Lund, S-220 07 Lund, Sweden

Received 20 May 1971

1. Introduction

It is generally accepted that the biosynthesis of polysaccharide proteins in mammalian tissues follows a certain schedule [1, 2] involving four principal steps: (a) formation of the polypeptide backbone, (b) stepwise addition of monosaccharide units from the appropiate nucleotide sugars to form the linkage region to protein, and (c) the alternate addition of hexosamine and uronic acid moieties from their respective UDP-sugars to the non-reducing terminus of the growing chain. Finally, (d) sulfation is accomplished by adding sulfate groups from 3′-phosphoadenosine-5′-phosphosulfate to certain of the monosaccharide moieties. The various glycosyltransferases involved are specific for the sugar residue to be transferred, as well as for the acceptor group. For example, transfer of *N*-acetylgalactosamine from UDP-GalNAc to oligosaccharides with glucuronic acid in the non-reducing terminus was catalyzed by a particulate enzyme preparation from embryonic chick cartilage [3]. However the same preparation failed to catalyze the transfer of *N*-acetylgalactosamine to an oligosaccharide with iduronic acid as the terminal sugar [4].

The formation of an *N*-acetylgalactosaminidic linkage to an L-iduronosyl residue constitutes a unique step in the biosynthesis of dermatan sulfate. It is the purpose of the present communication to demonstrate the transfer of *N*-acetylgalactosamin from UDP-GalNAc to an exogenous iduronosyl acceptor catalyzed by a particulate enzyme preparation from hog intestinal mucosa.

Abbreviations:
UDP :: Uridine-diphosphate
UDP-GalNAc: Uridine-diphospho-*N*-acetylgalactosamine
UDP-GlcUA : Uridine-diphospho-glucuronic acid
HEPES : *N*-2-hydroxypiperazine-*N*′-2-ethane sulfonic acid

2. Experimental

Hog intestine was obtained from the local slaughterhouse, immediately chilled on ice, rinsed with ice-cold saline and cut into pieces (length 35 cm). The mucosal surfaces were scraped with a metal spoon and the scrapings were homogenized in a Potter-Elvehjem homogenizer in 30 ml of a buffer of the following composition: Sucrose, 0.34 M; HEPES, pH 7.4, 50 mM; Na_2EDTA, 1 mM (buffer A). After centrifugation of the homogenate at 10,000 *g* for 20 min at 4° the supernatant liquid was subsequently centrifuged at 120,000 *g* for 2 hr at 4°. The resulting pellet was resuspended in buffer A and recentrifuged at 120,000 *g* for 1 hr. This procedure was repeated once and the final pellet was suspended in 1 ml of 10 mM HEPES, pH 7.2, and kept frozen until used. The oligosaccharide acceptor was prepared from decasaccharide fraction II: 2-18-4 in ref. [5] as follows. Pig skin dermatan sulfate was digested with hyaluronidase followed by gel chromatography of the low-molecular weight split products [5]. The decasaccharide material so obtained originated partly from internal segments and, partly, from the non-reducing terminal segment of the chains. Thus, the former

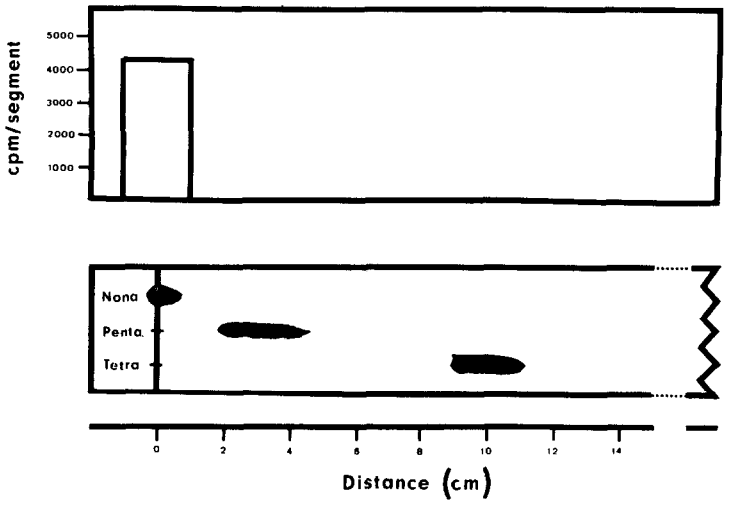

Fig. 1. Formation of labelled higher oligosaccharide from UDP-*N*-^{3}H-acetylgalactosamine and acceptor decasaccharide. For details, see text. Standards of nona-, penta-, and tetrasaccharides were obtained essentially as described elsewhere [9].

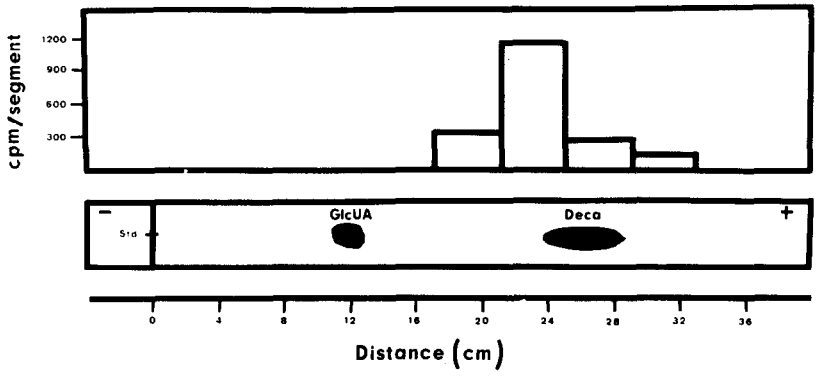

Fig. 2. Paper electrophoresis of undecasaccharide formed by transfer of *N*-acetylgalactosamine from UDP-*N*-^{3}H-acetylgalactosamine to an acceptor decasaccharide. The paper at the origin of the chromatogram in the assay procedure was eluted with water and the material (1969 cpm) was subjected to high voltage electrophoresis on Whatman 3 MM paper in pyridine–acetic acid buffer, pH 3.0 (44 V/cm, 1.5 hr). The paper was cut and the radioactivity was counted as described in the text. GlcUA and Deca, glucuronic acid and decasaccharide standards, respectively.

fragment contained non-reducing terminal glucuronic acid, while the latter fragment should contain non-reducing terminal iduronic acid. This material was subjected to exhaustive β-glucuronidase digestion followed by gel chromatography on a column (1.1 X 340 cm) of Sephadex G-50, superfine, essentially as described previously [6]. The undegraded decasaccharide material, which should contain iduronic acid in the non-reducing terminus had a carbazole-to-orcinol ratio of 0.44 [6]; the hexosamine content was 26.0% [7].

The incubation mixture for the assay of *N*-acetylgalactosamine transferase contained 88 μg of oligosaccharide acceptor (decasaccharide with non-reducing terminal iduronic acid) and (in micromoles): UDP-*N*-^{3}H-acetylgalactosamine, 0.012 (460,000 cpm); HEPES, pH 7.2, 2.0; $MnCl_2$, 0.20; NAD, 0.01; and microsomal enzyme (155 μg of protein) in a final

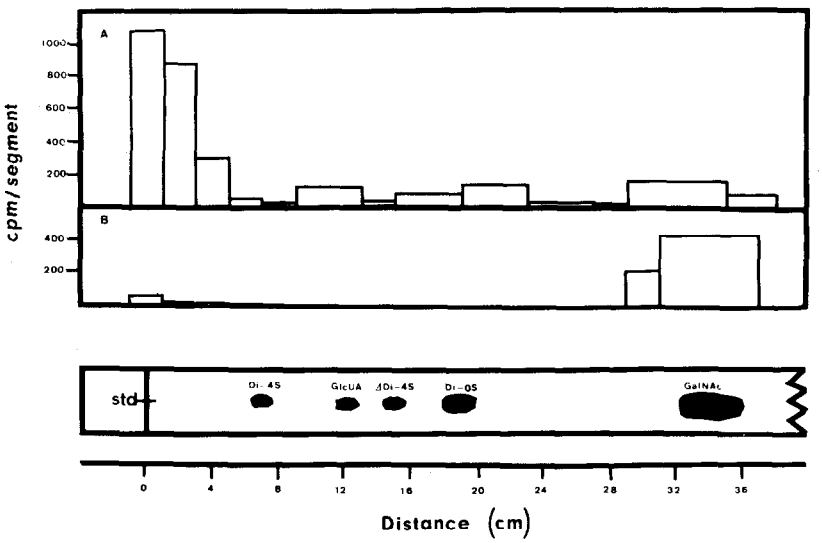

Fig. 3. Paper chromatography of digests of labelled undecasaccharide with chondroitinase-AC (A) and chondroitinase-ABC (B), respectively. Labelled undecasaccharide (4500 cpm) obtained as described in legend to fig. 2 was dissolved in 50 μl of 0.5 M tris, pH 8.0, and digested with 0.05 units of chondroitinase-AC [10] at 37° for 24 hr. The digestion mixture was evaporated to dryness, dissolved in 20 μl of water and subjected to descending paper chromatography on Whatmann 3 MM paper, which was irrigated with isobutyric acid–2 M ammonia (5:3) for 28 hr. The paper was cut and the radioactivity was counted as described in the text. Material remaining at the origin of this chromatogram (1080 cpm) was eluted with water and digested with chondroitinase-ABC. The digest was subjected to paper chromatography followed by radioactivity measurements as described above. Standards (std) of *N*-acetyl-chondrosine (Di-OS), saturated 4-sulfated disaccharide (Di-4S) and unsaturated 4-sulfated disaccharide (Δ Di-4S) were obtained as described elsewhere [9].

volume of 20 μl. After incubation at 37° for 1 hr, the total incubation mixture was streaked as a 1 cm band on a paper of Whatman 3 MM, which was developed in a descending system using 95% ethanol–1 M ammonium acetate pH 7.5 (7:3). The paper was cut and the strips were placed into vials containing 20 ml of scintillation mixture (4 g Omnifluor per liter of toluene) and counted in a TriCarb liquid scintillation counter.

3. Results and discussion

Incubation of UDP-GalNAc-^{3}H and decasaccharide with microsomal enzyme resulted in the formation of a labelled product with no mobility in the ethanol: ammonium acetate system as shown in fig. 1 (5307 cpm). Elution with water yielded 1969 cpm of radioactive material, which had a mobility slightly lower than that of a decasaccharide in paper electrophoresis (fig. 2). These data are in agreement with the formation of a labelled undecasaccharide containing an uncharged *N*-acetylgalactosamine moiety in non-reducing terminal position. It should be pointed out that the predominant charges would be 5 sulfate groups per 11 monosaccharide units in the labelled product, whereas the standard decasaccharide carries 5 sulfate groups per 10 monosaccharide units.

In order to confirm that the *N*-acetylgalactosamine moiety had been transferred to an L-iduronic acid residue digestions with chondroitinase-AC and -ABC, respectively, were performed. Treatment with chondroitinase-AC which cleaves hexosaminidic linkages to glucuronic acid [10] had virtually no effect on the oligosaccharide as shown in fig. 3A. However, treatment with chondroitinase-ABC which cleaves hexosaminidic linkages to both glucuronic and iduronic acid residues [10] liberated all of the radioactivity as *N*-acetylgalactosamine (fig. 3B). These experiments demonstrate, for the first time, the formation of an *N*-acetylgalactosaminidic linkage to an

L-iduronic acid residue. Characterization of the glycosyltransferase catalyzing this reaction will be of great interest for a more complete understanding of dermatan sulfate biosynthesis.

Acknowledgements

This investigation was supported by grants from the Swedish Medical Research Council (B70-13X-139-06B), the Gustaf V 80-års fond, The Alfred Österlund Foundation and the Medical Faculty of Lund. A.M. is recipient of a predoctoral scholarship ("Doktorand-stipendium") from the Medical Faculty, University of Lund. The expert technical assistance of Mrs. Lena Åberg and Birgitta Havsmark is gratefully acknowledged.

References

[1] A.C. Stoolmiller and A. Dorfman, in: Comprehensive Biochemistry, Vol. 17, eds. M. Florkin and E.H. Stotz (Elsevier, Amsterdam, 1969) p. 241.
[2] A. Dorfman, in: Chemistry and Molecular Biology of the Intercellular Matrix, Vol. 3, ed. E.A. Balazs (Academic Press, London and New York, 1970) p. 1421.
[3] A. Telser, H.C. Robinson and A. Dorfman, Arch. Biochem. 116 (1966) 458.
[4] A. Telser, Ph.D. Thesis (The University of Chicago, 1968).
[5] L.-Å. Fransson and L. Rodén, J. Biol. Chem. 242 (1967) 4170.
[6] L.-Å. Fransson and L. Rodén, J. Biol. Chem. 242 (1967) 4161.
[7] C.A. Antonopoulos, S. Gardell, J.A. Szirmai and E.R. De Tyssonsk, Biochim. Biophys. Acta 83 (1964) 1.
[8] J.E. Silbert, Methods Enzym. 8 (1966) 492.
[9] L.-Å. Fransson, L. Rodén and M.L. Spach, Anal. Biochem. 23 (1968) 317.
[10] T. Yamagata, H. Saito, O. Habuchi and S. Suzuki, J. Biol. Chem. 243 (1968) 1523.