DEMONSTRATION OF D-GLUCURONIC ACID AS REDUCING TERMINAL OF INTRACELLULAR HEPARAN SULFATES

Udo KLEIN and Kurt von FIGURA

Institute of Physiological Chemistry, University of Münster, D-4400 Münster, FGR

Received 20 September 1976

1. Introduction

Recent studies have revealed the existence of endoglycosidases specific for heparan-sulfate [1,2]. Heparan sulfate is split by these enzymes into oligosaccharides. The number of linkages susceptible to these endoglycosidases seems to be limited. Previous studies on the structure of heparan-sulfate chains isolated from fibroblasts of patients with heparan-sulfate-storing genetic mucopolysaccharidoses gave insight into the linkage type split by these endoglycosidases [3]. Heparan-sulfate chains that accumulate due to the deficiency of an exoglycosidase represent oligosaccharides with glucosamine and uronic acid residues as reducing terminals, thus suggesting the participation of endohexosaminidase(s) and endohexuronidase(s) in the degradation of heparan-sulfate. The present study extends these findings to the reducing terminals of heparan-sulfates that accumulate due to the deficiency of specific sulfatases. The demonstration of D-glucuronic acid as the only uronic acid present at the reducing terminals provides evidence for the existence of a heparan-sulfate-degrading endoglucuronidase.

2. Materials and methods

Fibroblast cultures from skin biopsies were established and maintained as described [4].

The intracellular [^{35}S]heparan-sulfates from Hunter fibroblasts (mucopolysaccharidosis II, sulfoiduronide sulfatase deficiency) and Sanfilippo A fibroblasts (mucopolysaccharidosis III A, heparan-sulfate sulfamidase deficiency) were isolated as described [3]. The intracellular [^{35}S]heparan-sulfates derived from Scheie (mucopolysaccharidosis I S, α-L-iduronidase deficiency) and Sanfilippo B (mucopolysaccharidosis III B, α-*N*-acetylglucosaminidase deficiency) fibroblasts were those as described [3].

The intracellular [^{35}S]heparan-sulfates were reduced with [^{3}H]$NaBH_4$ (NEN, Dreieichenhain) exactly as described [5]. After reduction the polysaccharides were precipitated with 80% ethanol–1% potassium acetate (final concentration). The precipitate was subjected to chromatography on a 1.5 × 35 cm Sephadex G-50 column (Pharmacia, Uppsala) in 0.2 M pyridine–acetic acid, pH 5.5 in order to remove acid-resistant ^{3}H-labelled low molecular material present in the commercially available [^{3}H]$NaBH_4$ preparations. The high molecular material was brought to dryness by evaporation.

[^{3}H]Xylitol [6], [^{3}H]galactitol [6], [^{3}H]glycosaminitol (see preparation of hexosamine fraction in ref. [7]) [8] and [^{3}H]aldonic acids [5] were identified as already described. [^{3}H]Idonic acid was prepared from L-iduronic acid isolated from the hydrolysate of dermatan sulfate by ion-exchange chromatography on Dowex 1 × 4 [10]. The lactones of [^{3}H]aldonic acids were prepared as described [9] and separated by chromatography on Selecta filter paper No. 2043 b (Schleicher Schüll, Dassel) in tert-amylalcohol-isopropyl alcohol-water (4:1:2, v/v/v) [5].

Liquid scintillation counting was performed in a 3390 Packard liquid scintillation counter, using Unisolve (Zinsser, Frankfurt) as the scintillation medium.

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Results and discussion

For analysis of the reducing-end groups the [^{35}S]heparan-sulfate chains were treated with [^{3}H]sodium borohydride, thus converting the reducing terminals into the corresponding sugar alcohols.

The hydrolysates of the polysaccharides were analyzed for the ^{3}H-derivatives of the sugars known to be present in heparan-sulfate, e.g. glucosamine, D-glucuronic acid, L-iduronic acid, galactose and xylose. The only ^{3}H-derivatives detectable were [^{3}H]glucosaminitol and [^{3}H]aldonic acids which were found in a ratio of 1.9:1 for heparan-sulfate chains from Sanfilippo A fibroblasts and 6.8:1 for heparan-sulfate chains from Hunter fibroblasts (table 1). These data are proof that glucosamine and uronic acid are reducing terminals and confirm the previous findings for intracellular heparan-sulfate stored in Sanfilippo B and Scheie fibroblasts, from which the existence of heparan-sulfate-degrading enzymes with the specificities of endoglucosaminidase(s) and endohexuronidase(s) were postulated [3].

D-Glucuronic acid and L-iduronic acid are the uronic acids present in heparan-sulfate. The type of uronic acid present at the reducing terminals of the heparan-sulfates that accumulate in the different mucopoly-saccharidoses was identified after lactonization of the [^{3}H]aldonic acid fraction. The only [^{3}H]lactone detectable after separation by paper chromatography was that of L-[^{3}H]gulonic acid (fig.1), the reduction product of D-glucuronic acid. The demonstration of

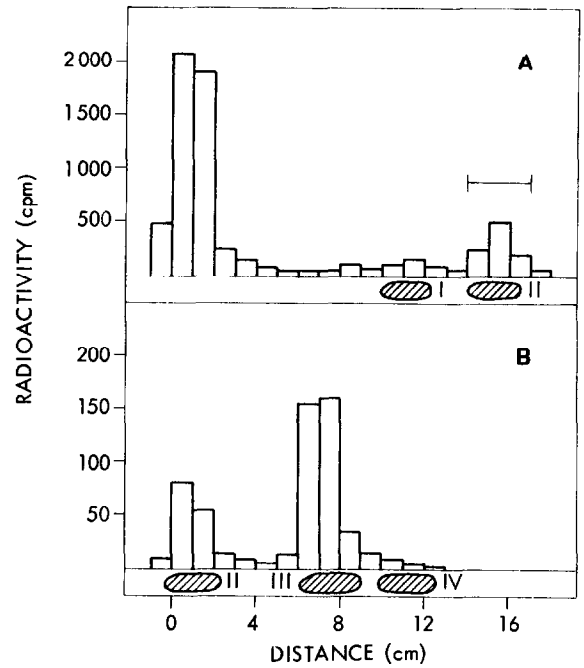

Fig.1. Identification of D-glucuronic acid as the reducing terminal of intracellular heparan-sulfate derived from Hunter fibroblasts. 1 A: Paper electrophoresis of the [^{3}H]aldonic acid fraction. Electrophoresis was performed at 75 V/cm for 45 min in 0.08 M pyridine–acetic acid, pH 5.3 [5]. 1 B: Paper chromatography of the lactonized [^{3}H]aldonic acid fraction obtained after high-voltage electrophoresis (fig.1A) in tert-amy alcohol/isopropyl alcohol/water (4:1:2, v/v/v) [5]. Reference [^{3}H]uronosyl anhydro-mannitol (I), a mixture of [^{3}H]idonic acid and [^{3}H]gulonic acid (II), [^{3}H]gulonolactone (III) and [^{3}H]idonolactone (IV). The lactonized [^{3}H]aldonic acid fractions obtained from intracellular heparan-sulfates derived from Scheie, Sanfilippo A and Sanfilippo B fibroblasts, behaved indentically to that of Hunter fibroblasts.

Table 1
Distribution of [^{3}H]sugar derivatives at the reducing-end of heparan-sulfates

	% of ^{3}H-Radioactivity			
Source of heparan-sulfate	[^{3}H]Xylitol	[^{3}H]Galactitol	[^{3}H]Glucosaminitol	[^{3}H]Aldonic Acid
Sanfilippo A	n.d.[a]	n.d.	65.4	34.6
Hunter	n.d.	n.d.	87.8	12.2

[a] n.d.: not detectable.

^{3}H-Radioactivity recovered as [^{3}H]glycosaminitol and [^{3}H]aldonic acid accounted for 67% (Sanfilippo A) and 78% (Hunter) of total ^{3}H-radioactivity and was taken as 100% for the calculations of this table. [^{3}H]Glucosaminitol was corrected for a loss of 15% during hydrolysis [3]. The residual ^{3}H-radioactivity was recovered in the neutral sugar fraction (prepared according to ref. [6]) and did not comigrate with xylitol or with galactitol.

D-glucuronic acid as the reducing terminal of intracellularly stored heparan-sulfate chains suggests that the heparan-sulfate-degrading endohexuronidase has the specificity of an endoglucuronidase. The existence of a heparan-sulfate-degrading endoglucuronidase is further supported by experiments with a partially purified endoglycosidase preparation from human placenta which liberates oligosaccharides from heparan-sulfate chains with D-glucuronic acid at the reducing terminal (manuscript in preparation).

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 104).

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