

Supplemental data

Xyloside-primed Chondroitin Sulfate/Dermatan Sulfate from Breast Carcinoma Cells with a Defined Disaccharide Composition has Cytotoxic Effects *In Vitro*

Andrea Persson, Emil Tykesson, Gunilla Westergren-Thorsson, Anders Malmström, Ulf Ellervik, and Katrin Mani

Tables S1 and S2

TABLE S1. **Disaccharide fingerprint of xyloside-primed GAGs and PG-GAGs from HCC70 cells and CCD-1095Sk cells.** Disaccharides from the indicated xyloside-primed GAGs, PG-GAGs, CS A, CS B, and CS C obtained after degradation with chondroitinase ABC, chondroitinase AC-I and –II, or chondroitinase B, followed by AMAC-labeling, and HPLC. The molar percent is calculated based on disaccharide standards of known quantity, and refers to the total molar amount of degraded material by the different enzymes. The percent of total is calculated based on the total amounts (in ng) of all of the disaccharides (including the heparinase I-III-generated ones). The data are means of two independent experiments \pm S.D., unless otherwise stated.

GAGs		Disaccharides (mol% \pm SD)						% of total
		Δ UA,2S-GalNAc,4S (B)	Δ UA-GalNAc,4S,6S (E)	Δ UA,2S-GalNAc,6S (D)	Δ UA-GalNAc,4S (A)	Δ UA-GalNAc,6S (C)	Δ UA-GalNAc (O)	
XylNapOH-primed GAGs 24 h HCC70	Chondroitinase ABC	-	2.8 \pm 0.4	0.25 \pm 0.1	14 \pm 0.8	81 \pm 3.6	-/3.5 ^a	93
	Chondroitinase AC	-	1.2 \pm 0.1	-	7.5 \pm 0.6	90 \pm 0.6	0.93 \pm 0.1	74
	Chondroitinase B	-	25 \pm 5.1	-	75 \pm 5.1	-	-	2.2
XylNapOH-primed GAGs 48 h HCC70	Chondroitinase ABC	-	2.6 \pm 0.5	0.38 \pm 0.1	14 \pm 0.9	83 \pm 1.3	0.17 \pm 0.1	80
	Chondroitinase AC	-	1.2 \pm 0.2	-	7.7 \pm 0.2	90 \pm 0.4	0.71 \pm 0.4	65
	Chondroitinase B	-	24 \pm 1.4	-	76 \pm 1.4	-	-	1.5
XylNapOH-primed GAGs 72 h HCC70	Chondroitinase ABC	-	2.8 \pm 0.4	-/0.23 ^a	16 \pm 0.1	81 \pm 0.2	0.49 \pm 0.3	75
	Chondroitinase AC	-	1.3 \pm 0.1	-	8.7 \pm 1.3	89 \pm 1.6	0.76 \pm 0.3	56
	Chondroitinase B	-	19 \pm 5.4	-	81 \pm 5.4	-	-	1.6
XylNap-primed GAGs HCC70	Chondroitinase ABC	-	3.0 \pm 0.3	0.56 \pm 0.1	15 \pm 0.6	81 \pm 1.1	0.55 \pm 0.2	65
	Chondroitinase AC	-	1.4 \pm 0.2	-	9.1 \pm 0.1	88 \pm 0.1	1.3 \pm 0.3	47
	Chondroitinase B	-	26 \pm 3.7	-	74 \pm 3.7	-	-	1.3
PG-GAGs untreated HCC70	Chondroitinase ABC	-	1.3 \pm 0.5	0.71 \pm 0.1	18 \pm 3.8	79 \pm 4.1	0.73 \pm 0.2	44
	Chondroitinase AC	-	-/0.74 ^a	-	11 \pm 1.2	87 \pm 2.6	1.3 \pm 0.9	34
	Chondroitinase B	-	-	-	100	-	-	0.39
XylNapOH-primed GAGs CCD-1095Sk	Chondroitinase ABC	1.5 \pm 0.1	0.24/1.3 ^a	3.4 \pm 0.7	56 \pm 5.8	38 \pm 4.6	0.86 \pm 0.3	98
	Chondroitinase AC	-	-	1.0 \pm 0.1	26 \pm 2.9	71 \pm 3.2	2.8 \pm 0.3	44
	Chondroitinase B	6.9 \pm 0.1	-	-	93 \pm 0.1	-	-	18
XylNap-primed GAGs CCD-1095Sk	Chondroitinase ABC	1.8 \pm 0.1	-/0.86 ^a	4.1 \pm 0.4	55 \pm 4.5	38 \pm 4.0	1.0 \pm 0.5	99
	Chondroitinase AC	-	-	1.3 \pm 0.2	25 \pm 4.7	70 \pm 6.5	3.0 \pm 1.6	38
	Chondroitinase B	7.7 \pm 1.0	-/1.2 ^a	-	92 \pm 1.8	-	-	16
PG-GAGs untreated CCD-1095Sk	Chondroitinase ABC	2.7 \pm 0.8	0.86 \pm 0.1	1.2 \pm 0.5	81 \pm 4.3	14 \pm 3.4	0.49 \pm 0.3	92
	Chondroitinase AC	-	-/0.86 ^a	-/0.97 ^a	57 \pm 3.8	41 \pm 4.3	1.7 \pm 0.8	35
	Chondroitinase B	7.2 \pm 1.6	-	-	93 \pm 1.6	-	-	36

GAGs		Disaccharides (mol% ± SD)						% of total
		ΔUA,2S-GalNAc,4S (B)	ΔUA-GalNAc,4S,6S (E)	ΔUA,2S-GalNAc,6S (D)	ΔUA-GalNAc,4S (A)	ΔUA-GalNAc,6S (C)	ΔUA-GalNAc (O)	
CS A from bovine trachea^b	Chondroitinase ABC	-	0.21	-	79	15	5.2	~100
	CS B from porcine intestinal mucosa^b	Chondroitinase ABC	1.6	3.6	0.22	89	4.4	1.0
	Chondroitinase B	2.6	3.9	-	93	-	-	60
CS C from shark cartilage^b	Chondroitinase ABC	-	-	-	5.4	92	2.3	~100

^a Variability between the different experiments. ^b Calculated based on one experiment.

TABLE S2. **Disaccharide fingerprint of xyloside-primed GAGs and PG-GAGs from HCC70 cells and CCD-1095Sk cells.** Disaccharides from the indicated xyloside-primed GAGs, PG-GAGs, and heparin obtained after degradation with heparinase I-III, followed by AMAC-labeling, and HPLC. The molar percent is calculated based on disaccharide standards of known quantity, and refers to the total molar amount of degraded material by the different enzymes. The percent of total is calculated based on the total amounts (in ng) of all of the disaccharides (including the chondroitinase ABC-generated ones). The data are means of two independent experiments \pm S.D., unless otherwise stated.

GAGs	Disaccharides (mol% \pm SD)								% of total
	Δ UA,2S-GlcNS,6S	Δ UA-GlcNS,6S	Δ UA,2S-GlcNS	Δ UA-GlcNS	Δ UA,2S-GlcNAc,6S	Δ UA-GlcNAc,6S	Δ UA,2S-GlcNAc	Δ UA-GlcNAc	
XylNapOH-primed GAGs 24 h HCC70	-/2.8 ^a	8.4 \pm 0.2	5.0 \pm 1.7	18 \pm 1.4	-	13 \pm 2.2	-	54 \pm 1.3	7.4
XylNapOH-primed GAGs 48 h HCC70	2.8 \pm 0.4	7.4 \pm 0.6	5.0 \pm 1.1	18 \pm 1.4	3.1 \pm 0.6	15 \pm 0.9	3.2 \pm 0.9	45 \pm 2.3	20
XylNapOH-primed GAGs 72 h HCC70	2.9 \pm 0.1	6.2 \pm 0.3	4.9 \pm 1.5	17 \pm 2.2	4.1 \pm 0.2	14 \pm 1.9	5.1 \pm 0.8	45 \pm 1.2	25
XylNap-primed GAGs HCC70	3.4 \pm 0.6	6.7 \pm 0.4	4.9 \pm 1.6	18 \pm 1.9	2.5 \pm 0.2	16 \pm 0.2	3.8 \pm 0.8	45 \pm 3.6	35
PG-GAGs untreated HCC70	2.2 \pm 0.3	4.3 \pm 0.4	3.3 \pm 2.2	19 \pm 2.9	1.7 \pm 0.3	11 \pm 0.8	3.7 \pm 0.5	55 \pm 4.3	56
XylNapOH-primed GAGs CCD-1095Sk	-	-	15 \pm 2.8	28 \pm 0.4	-	-	-	57 \pm 2.3	1.5
XylNap-primed GAGs CCD-1095Sk	-	-	17 \pm 1.7	27 \pm 2.4	-	-	-	56 \pm 4.1	1.3
PG-GAGs untreated CCD-1095Sk	-/1.9 ^a	4.1 \pm 0.4	4.1 \pm 0.3	23 \pm 0.6	-	11 \pm 0.6	-	60 \pm 6.3	8.5
Heparin from porcine intestinal mucosa^b	68	12	5.7	3.0	3.1	3.7	1.8	3.0	~100

^a Variability between the different experiments. ^b Calculated based on one experiment.