Production of marine chondroitin sulfate of defined molecular weight by enzymatic hydrolysis

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Motivation

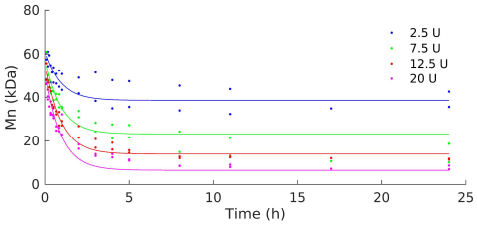
Chondroitin sulfate (CS) is a sulfated polysaccharide playing key roles in important biological processes such as cell differentiation. CS bioactivity is related to its ability to interact with proteins, which is in turn influenced by the sulfation pattern and molecular weight of the polymer [1]; hence modification of these characteristics appears attractive to tailor CS to particular applications. Besides characteristic sulfation, chondroitin sulfate from marine sources displays higher molecular weight than terrestrial counterparts [2], making it more amenable to produce CS in a wide range of molecular weights. Herein, we describe the depolymerization of CS from three cartilaginous fish species by enzymatic hydrolysis with hyaluronidase and chondroitinase ABC to establish the conditions of reaction to produce CS of defined molecular weights.

Materials and methods

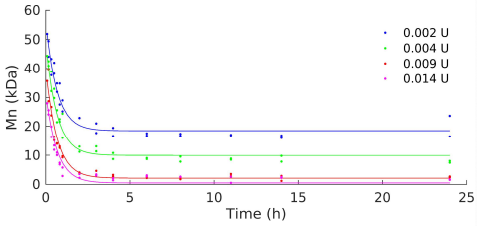
Chondroitin sulfate from shark (*Prionace glauca*), ray (*Raja clavata*) and chimaera (*Chimaera monstrosa*) was depolymerized with chondroitinase ABC from *Proteus vulgaris* (0.002, 0.004, 0.009 and 0.014 units / mg CS) and hyaluronidase from bovine testes (2.5, 7.5, 12.5 and 20.0 units / mg CS). Reactions were carried out in 50mM TRIS-HCl / 150mM NaAcO at pH 8 and 5mM NaH_2PO_4 / 150mM NaCl at pH 4 respectively. At various times, aliquots were taken and reactions stopped by heating at 70ºC for 25 min. After centrifugation, 100 µl of supernatant was filtered through 0.2 µm PES membranes and injected onto a GPC system (Agilent) equipped with a set of four PSS Suprema columns (precolumn 5µm, 8x50mm; 30Å 5µm, 8x300mm; 100Å 5µm, 8x300mm; and ultrahigh 10µm, 8x300mm). Absolute molecular weights were estimated combining refractive index and dual angle light scattering signals using refractive index increments (dn/dc) of 0.110

Depolymerization kinetics and molecular weight distributions

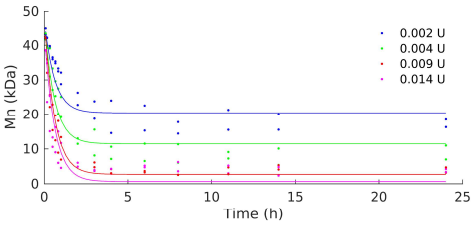
Hyaluronidase (*Shark*)



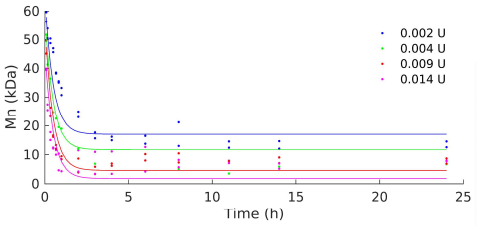
Chondroitinase (*Shark*)



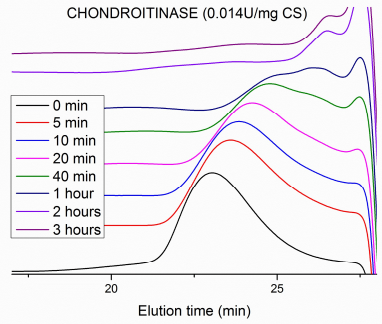
Chondroitinase (*Ray*)

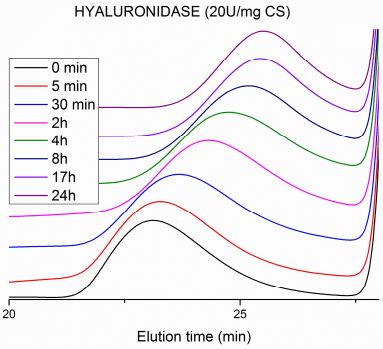


Chondroitinase (*Chimaera*)



Gel permeation chromatography (GPC)





Shark CS (*P. Glauca*)

$$\frac{dM_n}{dt} = -r[M_n - A\exp(-\mu R)]$$

M_n: Number average molecular weight (kDa)
t: time of CS hydrolysis (h)
E: enzyme to substrate ratio (U enzyme/mg CS)
r: specific rate of CS hydrolysis (h^{-1})
A: fitting parameter (kDa)
μ: fitting parameter (mg CS/U enzyme)
$\bar{\varepsilon}$: mean fitting error (kDa)

	r	A	µ	$\bar{\varepsilon}$	R^2_{adj}
Hyaluronidase (shark)	1.07	49.82	0.10	2.63	0.943
Chondroitinase (shark)	1.50	34.29	310	1.29	0.985
Chondroitinase (ray)	1.65	36.38	289	2.59	0.940
Chondroitinase (chimaera)	2.05	24.74	187	4.01	0.884

Conclusions

We demonstrate that depolymerization kinetics for both enzymes can be fitted to a single mathematical form and calculate the parameters corresponding to each experiment. The resulting equations allow to estimate combinations of enzyme to substrate ratio and time required to obtain CS of defined molecular weight.

References

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2. J. Valcarcel, R. Novoa-Carballal, R. I. Pérez-Martín, R. L. Reis and J. A. Vázquez, Glycosaminoglycans from marine sources as therapeutic agents, Biotechnol Adv, 35(6):711-25 (2017).

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