SUPPLEMENTARY DATA

for

Oxidation of heparan sulfate by hypochlorite: role of *N*-chloro derivatives and dichloramine-dependent fragmentation

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Running title: HOCl-induced damage to heparan sulfate

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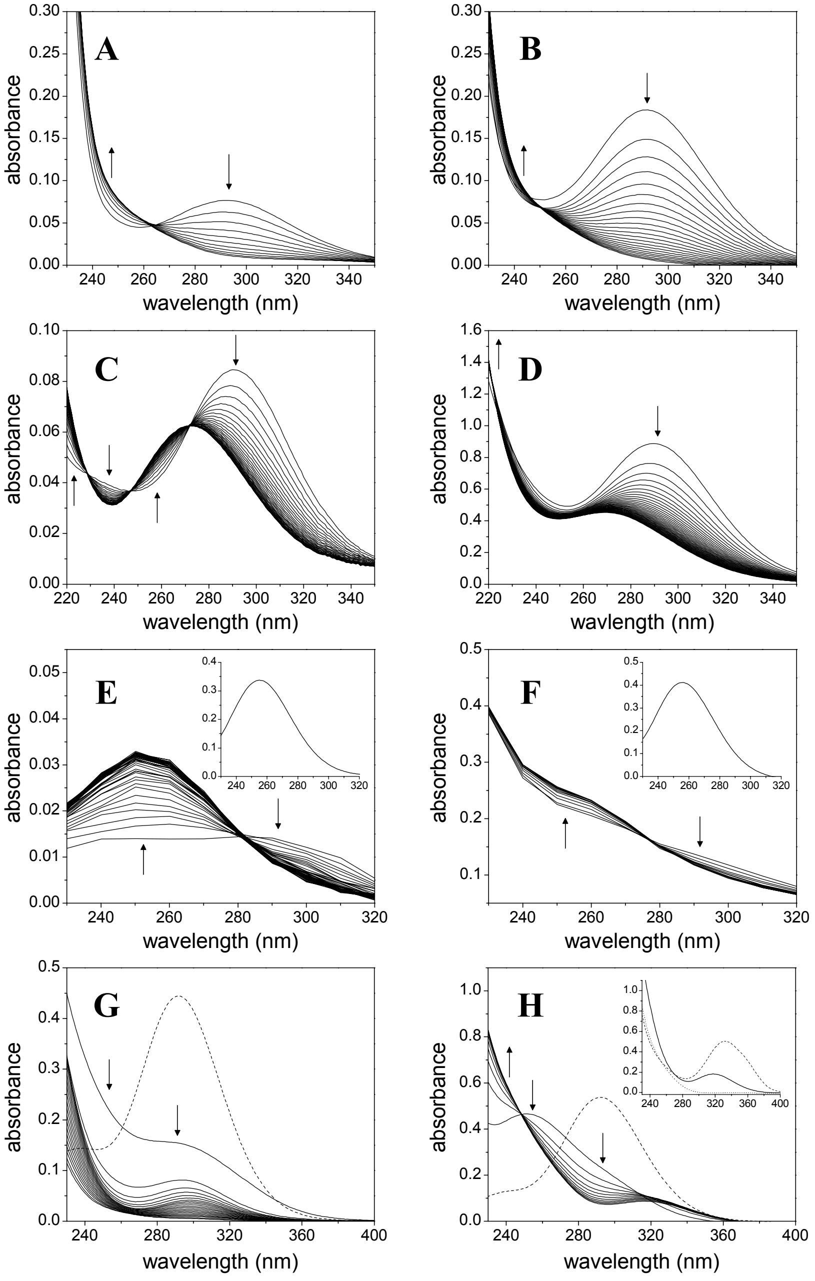
Supplementary Data Table 1: **Parameters used for modelling the reaction of HOCl with proteoglycans**

	Component	Glypican-1 [a]	Perlecan [a]	k_2 (22°C) M^{-1} s^{-1} [b]
Core protein	Met	10	41	3.8×10^7
	Cys	2	84	3.0×10^7
	Cystine	7	52	1.6×10^5
	His	9	165	1.0×10^5
	Lys	20	68	5.0×10^3
	Trp	6	40	1.1×10^4
	Tyr	11	109	44
	Arg	41	258	26
	Asn	19	107	0.03
	Glc	26	254	0.03
	Terminal amine	1	1	1.0×10^5
	Backbone amide	506	4369	0.11
Heparan sulfate chains	GlcNAc	171	171	0.0108
	$GlcNSO_3$	117	117	0.062
	$GlcNH_2$	12	12	7.6×10^5
	($GlcNH_2$ chloramine)	(0)	(0)	14

[a] Abundance data derived as detailed in the Experimental section. [b] Second-order rate constants for reaction with proteoglycan components are those previously reported for protein components [17] and those reported here for glycosaminoglycan components, as detailed in the Experimental section.



Supplementary Data Figure 1: UV-vis spectroscopy data for reaction of HOCl with glycosamine monosaccharides and glycosaminoglycans at pH 7.4. (**A**) Formation of GlcNAc chloramides on reaction of GlcNAc (20 mM) with HOCl (0.5 mM) at 37 °C; spectra recorded from t = 0.5 – 41.75 min at 3.75 min intervals. (**B**) Formation of polymer-derived GlcNAc chloramides on reaction of HA (8.08 mg ml^{-1}; 20 mM GlcNAc residues) with HOCl (1 mM) at 37 °C; spectra recorded from t = 0.5 – 900.5 min at 5 min intervals. (**C**) Formation of $GlcNSO_3$

N-chlorosulfonamides on reaction of $GlcNSO_3$ (2.5 mM) with HOCl (0.5 mM) at 22 °C, spectra recorded from t = 0.5 – 12.5 min at 0.75 min intervals. (**D**) Formation of polymer-derived $GlcNSO_3$ *N*-chlorosulfonamides on reaction of *b*-HP (6.4 mg ml^{-1}; 9.67 mM $GlcNSO_3$, 0.23 mM GlcNAc) with HOCl (5 mM) at 37 °C; spectra recorded from t = 0.5 – 180.5 min at 5 min intervals. (**E**) Formation of $GlcNH_2$ chloramines on reaction of $GlcNH_2$ (0.25 mM) with HOCl (0.1 mM) at 9.5 °C spectra recorded from t = 2.5 ms – 100.5 ms at 2 ms intervals. Inset: spectrum of $GlcNH_2$ (1 mM) with HOCl (1 mM) at 22 °C after t = 0.5 min. (**F**) Formation of polymer-derived $GlcNH_2$ chloramines on reaction of *dnsp*-HP (6.4 mg ml^{-1}; 1.32 mM $GlcNH_2$ residues) with HOCl (0.2 mM) at 10 °C; spectra recorded from t = 2.5 – 62.5 ms at 5 ms intervals. Inset: difference spectrum obtained by subtracting the spectrum of *dnsp*-HP alone (6.4 mg ml^{-1}) from the spectrum of *dnsp*-HP (6.4 mg ml^{-1}; 1.32 mM $GlcNH_2$ residues) after reaction with HOCl (1.32 mM) at 22 °C for t = 0.5 min. (**G**) Formation, and subsequent decomposition, of $GlcNH_2$ dichloramines formed on reaction of $GlcNH_2$ (1 mM) with HOCl (2.5 mM) at 22 °C spectra recorded from t = 0.5 – 45.5 min at 2.25 min intervals. Spectrum indicated by dashed line is HOCl alone (2.5 mM). (**H**) Formation of polymer-derived $GlcNH_2$ dichloramines and subsequent decomposition products. Difference spectra obtained by subtracting the spectrum of *dnsp*-HP alone (6.4 mg ml^{-1}) from spectra of *dnsp*-HP (6.4 mg ml^{-1}; 1.32 mM $GlcNH_2$ residues) treated with HOCl (2.64 mM) at 37 °C spectra recorded from t = 0.5 – 20.5 min at 2 min intervals. Spectrum indicated by dashed line is HOCl alone (2.64 mM). Inset: difference spectra obtained by subtracting the spectrum of *dnsp*-HP alone (6.4 mg ml^{-1}) from *dnsp*-HP (6.4 mg ml^{-1}; 1.815 mM $GlcNH_2$ residues) treated with HOCl (3.63 mM) at 37 °C, with spectra recorded at 0.75 h (solid line), 6 h (dotted line) and 24 h (dashed line). In each case arrows indicate direction of changes in spectral intensity over time.