SUPPLEMENTAL INFORMATION

Carbohydrate-Carbohydrate Interactions Mediated by Sulfate Esters and Calcium Provide the Cell Adhesion Required for the Emergence of Early Metazoans

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List of supplemental data

Table S-1. ^{1}H and ^{13}C chemical shifts (ppm) for residue of α-glucose in DaSP and standard compounds.

Unit	A	B	C	F	G	Standard compounds			
Proposed structure	→3-α-Glc1→	→3-α-Glc-2,4 di (SO_3^-)1→	→3,4-α-Glc1→	→3-α-Glc-4 (SO_3^-)1→	→3-α-Glc1→	α-Glc-O-Me[a]	→3-α-Glc1→[a]	→3-α-Glc-2,4,6 tri(SO_3^-)1→[b,c]	→3-α-Glc-2,4,6 tri(SO_3^-)1→[d,c]
H1/C1	5.33/100.06	5.7/97.06	5.33/99.15	5.28/99.71	5.40/98	4.81/100.19	5.33/100.6	97.1	99.0
H2/C2	3.64/70.06	**4.25/76.49**	3.70/71.30	3.70/71.19	3.79/70.91	3.56/72.23	3.68/71.26	**77.4**	**78.3**
H3/C3	**3.87/81.52**[a]	**3.95/80.01**	**3.94/80.87**	**3.89/80.83**	**3.83/80.79**	3.69/74.10	**3.90/81.32**	**81.4**	**83.7**
H4/C4	3.72/70.42	**4.23/76.49**	**3.98/77.88**	**4.22/76.61**	3.61/70.83	3.41/70.68	3.69/70.71	**75.4**	**76.2**
H5/C5	3.98/72.13	4.20/72.50	4.07/71.96	3.99/72.13		3.64/75.52	3.99/72.58	71.6	74.1
H6/C6	~3.76-3.85/61.2	~3.70-3.8/60.69	~3.76-3.85/61.2		~3.76-3.85/61.2	3.64-3.87/61.87	3.78-3.85/61.36	**67.1**	**69.6**

[a] Data from Lundborg and Widmalm (41).

[b] Data from Huang and Zhang (42).

[c] ^{13}C chemical shifts.

[d] Data from Bao et al (43).

Glycosylation and sulfation sites in blue and red, respectively.

Table S-2. ^{1}H and ^{13}C chemical shifts (ppm) for residue of α-fucose in DaSP and standard compounds.

Unit	D	Standard Compounds		
Proposed structure	→3-α-Fuc-2 (SO_3^-)1→	→3-α-Fuc-2 (SO_3^-)1→[a]	→3-α-Fuc1→[b]	→4-α-Fuc1→[c]
H1/C1	5.46/96.34	5.33/96.6	5.03/98.23	5.07/103.50
H2/C2	**4.41/73.45**	**4.55/75.56**	3.96/69.05	3.95/71.35
H3/C3	**4.10/70.69**	**4.10/75.9**	**4.01/77.66**	4.08/67.69
H4/C4	3.93/72.68	4.07/71.20	3.96/71.11	**3.97/82.75**
H5/C5	4.23/70.9	4.42/68.60	4.52/69.05	4.58/70.02
H6/C6	1.23/16	1.25/17.30	1.21/17.90	1.37/13.00

[a] Data from Pereira et al (44).

[b] Data from Alves et al (45).

[c] Data from Vilela-Silva et al (40).

Glycosylation and sulfation sites in blue and red, respectively.

Table S-3. ^{1}H and ^{13}C chemical shifts (ppm) for residue of α-galactose in DaSP and standard compounds.

Unit	E	Standard compounds			
Proposed structure	α-Gal1→	→4-α-Gal1→[a]	→3-α-Gal1→[b]	α-Gal-O-Me[c]	α-Gal-4,6-Py1→[c]
H1/C1	5.11/99.35	5.03/101.3	5.26/98.1	4.85/100.32	5.12/96.52
H2/C2	3.74/69.18	3.84/70.0	4.08/73.5	3.84/69.17	3.93/68.56
H3/C3	4.08/68.90	3.95/70.0	**4.14/77.2**	3.81/70.46	3.96/68.38
H4/C4	**3.99/70.01** **4.43/68.95**[d]	**4.13/79.8**[d]	4.32/69.5	3.99/70.19	**4.20/71.90**
H5/C5	Nd	4.34/70.0	4.24/68.2	3.89/71.54	3.91/62.97
H6/C6	**3.80-3.95/61.5**	3.81/61.5	3.82/63.9	3.76/62.06	**3.82-4.00/65.17**

[a] Data from Pavão et al (46).

[b] Data from Alves et al (45).

[c] Data from Lundborg and Widmalm (41).

[d] Pyruvated site.

Glycosylation and pyruvated sites in blue and green, respectively.

Table S-4. Contact points between residues of DaSP based on ^{13}C-^{1}H HMBC spectrum.

Inter-residue contact	Value in ppm (C-H)
E ($^{13}C1$) - D ($^{1}H3$)	99.35-4.10
D ($^{13}C1$) - F ($^{1}H3$)	96.34-3.89
F ($^{13}C1$) - G ($^{1}H3$)	99.71-3.83
G ($^{13}C1$) - C ($^{1}H3$)	98-3.98
C ($^{13}C1$) - B ($^{1}H3$)	99.15-3.95
A ($^{13}C1$) - A ($^{1}H3$)	99.15-3.87
B ($^{13}C1$) - A ($^{1}H3$)	97.06-3.87
A ($^{13}C1$) - B ($^{1}H3$)	100.06-3.95
A ($^{13}C1$) - C ($^{1}H3$)	100.06-3.94

Table S-5. ^{1}H and ^{13}C chemical shifts (ppm) of the correlation peaks, δH/ δC (ppm), of pyruvate involved in cyclic ketals with non-reducing terminal galactose derived from the $^{1}H/^{13}C$ HMBC spectra.

Chemical group	Pyruvated galactose in DaSP	Reference values		
		Six-membered ring (O-4 and O-6 substituted)	Five-membered ring (O-3 and O-4 substituted)	
CH_3	1.48/25.3	1.45/25.49[a]	1.62/26.4[b]	1.77/23.4[c]
O-C-O	1.48/101.2	1.45/102.10	1.62/108.3	1.77/109.1
COOH	1.48/175.2	1.45/175.2	1.62/178.5	1.77/186.6

[a] Data from Lundborg and Widmalm (41) for pyruvate linked to terminal α-galactose.

[b] Data from Bilan et al (47).

[c] Data from Farias et al (48).

Table S-6. AFM DFS data for the self-interactions of native (Nat), carboxyl-reduced (CR) and desulfated (Des) DaSP in the presence (Ca^{++}) and absence (EDTA) of calcium.

	k_{off} (s^{-1})[a,b]	X_β (Å)[c,b]	τ (s)[d]
Nat/Ca^{++}	1.39 ± 1.22 x 10^{-3}	3.65 ± 0.51	718.8
Nat/EDTA	0.41 ± 0.29	2.10 ± 0.16	2.4
CR/Ca^{++}	1.37 ± 1.18 x 10^{-3}	3.49 ± 0.59	729.9
CR/EDTA	0.39 ± 0.20	2.15 ± 0.17	2.5
Des/Ca^{++}	0.36 ± 0.23	2.40 ± 0.25	2.7
Des/EDTA	0.40 ± 0.28	2.22 ± 0.18	2.5

[a] k_{off} = thermal off-rate constant under zero external load.

[b] Data expressed as mean ± SD.

[c] x_β = reaction length.

[d] τ = mean lifetime of the complex.