Supplementary Materials

Novel Ca^{2+}-independent carbohydrate recognition of the C-type lectins, SPL-1 and SPL-2, from the bivalve *Saxidomus purpuratus*

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Table S1. PCR primers for amplification of the SPL cDNA.

Primer	Nucleotide sequence	Application
Forward		
DF1	5′- CCIWSIGGITGGAARTTYTTYGG-3′	Degenerate primer for SPL B-chain
F1	5′-GGTCCTGTTATCTTTTTGTGAA-3′	3′-RACE for SPL B-chain
F2	5′-ATGCTGCAAATGCGATTGTCAA-3′	3′-RACE for SPL A-chain
F3	5′-CTACCTATTCGACGAGACAGAA-3′	3′-RACE for SPL A-chain
F4	5′-GCCTTGGTGACTGTTGAAAGTTCAG-3′	3′-RACE for SPL A-chain
F5	5′-GTCATTTCAGCTCAGAGTGCTTTT-3′	3′-RACE for SPL A-chain
Reverse		
DR1	5′- RAAIGCYTTISWICCYTCCCAICC-3′	Degenerate primer for SPL B-chain
R1	5′-ATCAGAAAGATAACAGGAGCCA-3′	5′-RACE for SPL B-chain
R2	5′-TCAGTCAGAACAATCTATTTCGC-3′	5′-RACE for SPL B-chain
R3	5′-CCGATATTCGCTATGCTCTGCATCCCAGCTTC-3′	5′-RACE for SPL A-chain
R4	5′-AGATAGAGGACCCATCAATCCAC-3′	5′-RACE for SPL A-chain

Table S2. Data collection and refinement statistics.

Crystal type	SPL-1 (Pb-SAD)	SPL-1	SPL-2/GalNAc
Data collection and processing statistics			
Space group	$P2_1$	$P2_1$	$P3_121$
Unit cell dimension (Å)			
a (Å)	43.5	44.3	91.2
b (Å)	72.8	72.6	91.2
c (Å)	44.9	45.1	48.8
β (°)	94.4	95.3	120
Wavelength (Å)	1.5418	1.000	1.000
Resolution (Å)	44.7-2.50 (2.64-2.50) [a]	50.0-1.60 (1.63-1.60)	50.0-2.00 (2.03-2.00)
Total reflections	194781	196109	175796
Unique reflections	9724	37306	16065
$I/\sigma I$	20.5 (4.4)	17.8 (2.7)	39.6 (5.7)
Redundancy	20.0 (20.0)	5.3 (5.0)	10.9 (10.4)
Completeness (%)	99.8 (99.4)	99.3 (98.3)	99.9 (100)
R_{merge} [b] (%)	11.6 (68.8)	9.6 (61.6)	6.8 (46.2)
$CC_{1/2}$	0.999 (0.957)	0.990 (0.816)	0.999 (0.984)
HA sites	6		
Refinement statistics			
Resolution		38.2-1.60	30.7-2.00
Protein atoms		2210	1106
Ligand atoms		2	24
Water molecule		584	104
R_{work}/R_{free}(%)		20.3 / 24.5	17.8 / 20.8
Root mean square deviations			
Bond lengths (Å)		0.002	0.015
Bond angles (°)		0.840	1.922
Ramachandran statistics (%)			
Residues in favored region		95.9	96.3
Residues in allowed region		3.7	3.7
Residues in outlier region		0.4	0.0

[a]The values in parentheses are for the highest resolution shell.

[b]$R_{merge} = 100\Sigma|I - \langle I\rangle|/\Sigma I$, where I is the observed intensity and $\langle I\rangle$ is the average intensity of multiple observations of symmetry-related reflections.

Table S3. Glycan structures.

No.	Glycan
1	β-Glc-Sp
2	β-Gal-Sp
3	α-Man-Sp
4	α-Fuc-Sp
5	α-Rha-Sp
6	β-GlcNAc-Sp
7	β-GalNAc-Sp
8	Tobramycin
9	Gal-β-1,3-GlcNAc-β-Sp
10	Gal-α-1,3-Gal-β-1,3-GlcNAc-β-Sp
11	Neu5Ac-α-2,3-Gal-β-1,3-GlcNAc-β-Sp
12	Neu5Ac-α-2,6-Gal-β-1,3-GlcNAc-β-Sp
13	Neu5Gc-α-2,3-Gal-β-1,3-GlcNAc-β-Sp
14	Neu5Gc-α-2,6-Gal-β-1,3-GlcNAc-β-Sp
15	Gal-β-1,3-(Fuc-α-1,4)-GlcNAc-β- [Lewis A] –Sp
16	Gal-β-1,4-Glc-β-Sp
17	Gal-α-1,3-Gal-β-1,4-Glc-β-Sp
18	Gal-α-1,4-Gal-β-1,4-Glc-β-Sp
19	GlcNAc-β-1,3-Gal-β-1,4-Glc-β-Sp
20	GalNAc-β-1,3-Gal-β-1,4-Glc-β-Sp
21	Neu5Ac-α-2,3-Gal-β-1,4-Glc-β-Sp
22	Neu5Ac-α-2,6-Gal-β-1,4-Glc-β-Sp
23	Neu5Gc-α-2,3-Gal-β-1,4-Glc-β-Sp
24	Neu5Ac-α-2,6-Gal-β-1,4-Glc-β-Sp
25	Gal-β-1,4-(Fuc-α-1,3)-Glc-β-Sp
26	GalNAc-β-1,3-Gal-α-1,4-Gal-β-1,4-Glc-β-Sp
27	GlcNAc-β-1,6-GlcNAc-β-Sp
28	4-P-GlcNAc-b-1,4-Man-b-Sp
29	Glc-α-1,2-Gal-α-1,3-Glc-α-Sp

30 Gal-β-1,3-GalNAc-α-Sp

31 Gal-β-1,4-GlcNAc-β-Sp

32 Gal-β-1,4 -(Fuc-α-1,3)-GlcNAc-β- [Lewis X] –Sp

33 Neu5Ac-α-2,3-Gal-β-1,4-(Fuc-α-1,3)-GlcNAc-β- [Sialyl Lewis X]-Sp

34 Neu5Ac-α-2,3-Gal-β-1,3 -(Fuc-α-1,4)-GlcNAc-β- [Sialyl Lewis A]-Sp

35 Neu5Gc-α-2,3-Gal-β-1,3-(Fuc-α-1,4)-GlcNAc-β- [Sialyl Lewis A]-Sp

36 Gal-α-1,4-Gal-β-1,3-GlcNAc-β-Sp

37 Gal-β-1,4-GlcNAc-β-1,3-Gal-β-1,4-Glc-β- [LNnT]-Sp

38 GlcA-β-1,4-GlcNAc-α-1,4-GlcA-β-Sp

39 GlcNAc-β-1,6-(Gal-β-1,3)-GalNAc-α-O-Ser-Sp4

40 Neu5Ac-α-2,3Gal-β-1,4-(6S)GlcNAc-β-Sp

41 GalNAc-β-1,4-GlcNAc-β-Sp2

42 Neu5Ac-α-2,8-Neu5Ac-α-2,3-Gal β-1,4-Glc-β-Sp

43 Neu5Gc-α-2,8-Neu5Ac-α-2,3-Gal-β-1,4-Glc-β-Sp

44 GalNAc-α-1,3-(Fuc-α-1,2)-Gal-β-1,4-Glc-β- [Blood A antigen tetrose]-Sp1

45 GlcNAc-β-1,2-Man-α-Sp

46 Neu5Ac-α-2,3-Gal-β-Sp1

47 Gal-β-1,3 -GalNAc-β-1,3-Gal-β-Sp1

48 Glc-α-1,2-Gal-α-Sp

49 Gal-β-1,4-(Fuc-α-1,3)-GlcNAc-β-1,3-Gal-β-Sp1

50 Neu5Ac-α-2,3-Gal-β-1,4-(Fuc-α-1,3)-Glc-β- [3-Sialyl-3-fucosyllactose/ F-SL]-Sp1

51 GlcNAc-β-1,4-GlcNAc-β-Sp1

52 β-D-GlcA-Sp

53 Gal-β-1,4-(6S)GlNAc-β-Sp

54 GlcNAc-α-1,3-(Glc-α-1,2-Glc-α-1,2)-Gal-α-1,3-Glc-α-Sp

55 Gal-β-1,3-GalNAc-β-1,4-(Neu5Gc-α-2,3)-Gal-β-1,4-Glc-β-Sp1

56 Sisomicin Sulfate

57 GalNAc-α-1,3-(Fuc-α-1,2)-Gal-β- [Blood A antigen trisaccharide]-Sp1

58 Fuc-α-1,2-Gal-β-1,4-GlcNAc-β- [Blood H antigen trisaccharide]-Sp1

59 Gal-α-1,3-(Fuc-α-1,2)-Gal-β- [Blood B antigen trisaccharide]-Sp1

60 Fuc-α-1,2-Gal-β-1,3-GlcNAc-β-1,3-Gal-β-1,4-Glc-β- [LNFP I]-Sp1

61 Fuc-α-1,2-Gal-β-1,4-Glc-β- [Blood H antigen trisaccharide]-Sp1

62 Gal-α-1,3-(Fuc-α-1,2)-Gal-β-1,4-Glc-β- [Blood B antigen tetrasaccharide]-Sp1

63 (Fuc-α-1,2)-Gal-β-1,4-(Fuc-α-1,3)-GlcNAc-β- [Lewis Y]-Sp1

64 (Fuc-α-1,2)-Gal-β-1,3-(Fuc-α-1,4)-GlcNAc-β- [Lewis B]-Sp1

65 Gal-β-1,3-(Fuc-α-1,4)-GlcNAc-β-1,3-Gal-β-1,4-(Fuc-α-1,4)-Glc-β- [Lewis A]-Sp1

66 Gal-β-1,3-GalNAc-β-Sp1

67 Gal-β-1,3-(Neu5Ac-α-2,6)-GalNAc-β-Sp

68 Neu5Ac-α-2,6-Gal-β-1,3-GalNAc-β-Sp

69 Neu5Ac-α-2,6-Gal-β-1,3-(Neu5Ac-α-2,6)-GalNAc-β-Sp

70 Neu5Ac-α-2,3-Gal-β-1,3-(Neu5Ac-α-2,6)-GalNAc-β-Sp

71 Neu5Ac-α-2,6-(Neu5Ac-α-2,3)-Gal-β-1,3-GalNAc-β-Sp

72 GalNAc-β-1,4-(Neu5Ac-α-2,3)-Gal-β-1,4-Glc-β- [GM2]-Sp

73 GalNAc-β-1,4-(Neu5Ac-α-2,8-Neu5Ac-α-2,3)-Gal-β-1,4-Glc-β- [GD2]-Sp

74 Gal-α-1,4-Gal-β-1,4-GlcNAc-β-Sp1

75 β-D-Rha-Sp

76 Glc-α-1,4-Glc-β-Sp1

77 Glc-α-1,6-Glc-α-1,4-Glc-β-Sp1

78 Maltotriose-β-Sp1

79 Glc-α-1,6-Glc-α-1,6-Glc-β-Sp1

80 Maltotetraose-β-Sp1

81 GlcNAc-α-1,4-GlcA-β-1,4-GlcNAc-α1,4-GlcA-β-Sp

82 Maltohexaose-β-Sp1

83 Maltoheptaose-β-Sp1

84 Acarbose-β-Sp1

85 D-pentamannuronic acid-β-Sp1

86 L-pentaguluronic acid-β-Sp1

87 D-cellose-β-Sp1

88 Gal-α-1,3-Gal-β-Sp1

89 β-1,4-Xylotetrose-Sp1

90 Chitin-trisaccharide-Sp1

91 KDN-α-2,8-Neu5Ac-α-2,3-Gal-β-1,4-Glc-β-Sp

92 Neu5Ac-α-2,8-Neu5Gc-α-2,3-Gal-β-1,4-Glc-β-Sp

93 Neu5Ac-α-2,8-Neu5Ac-α-2,8-Neu5Ac-α-2,3-Gal-β-1,4-Glc-β-Sp3

94 Neu5Ac-α-2,8-Neu5Ac-α-2,6-Gal-β-1,4-Glc-Sp5

95 Gal-β-1,3-GalNAc-β-1,4-(Neu5Ac-α-2,3)-Gal-β-1,4-Glc-β-Sp1

96	Gentamicin Sulfate
97	Kanamycin sulfate
98	Geneticin Disulfate Salt (G418)
99	Neomycin trisulfate
100	SGP

Linkers

Sp: $OCH_2CH_2CH_2NH_2$

Spl: $NH(CH_3)OCH_2CH_2NH_2$

Sp2: $OCH_2CH_2NH_2$

Sp3:$O(CH_2)_3NHCOCH_2(OCH_2CH_2)_5CH_2CH_2NH_2$

Sp4: $OCH_2CH(COOH)NH_2$

Sp5: 2-amino-N-(2-amino-ethyl)-benzamide

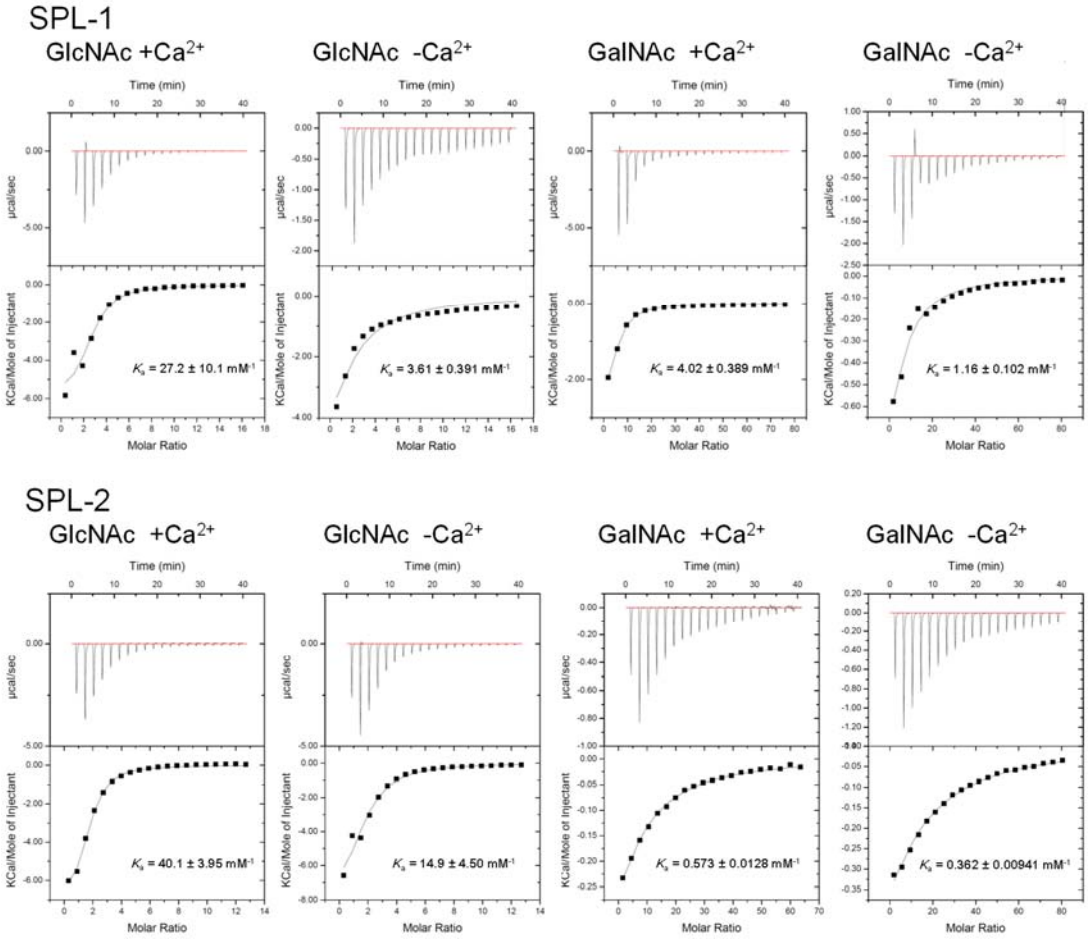

Figure S1. Binding of the carbohydrates to SPLs measured by ITC. The upper panels show raw data obtained for the injections of the carbohydrate solutions. The lower panels show integration of the peaks after correction for the heat of dilution with the best fitting curves by one-set of sites model. Association constants (K_a) for the binding of the carbohydrates are also shown.

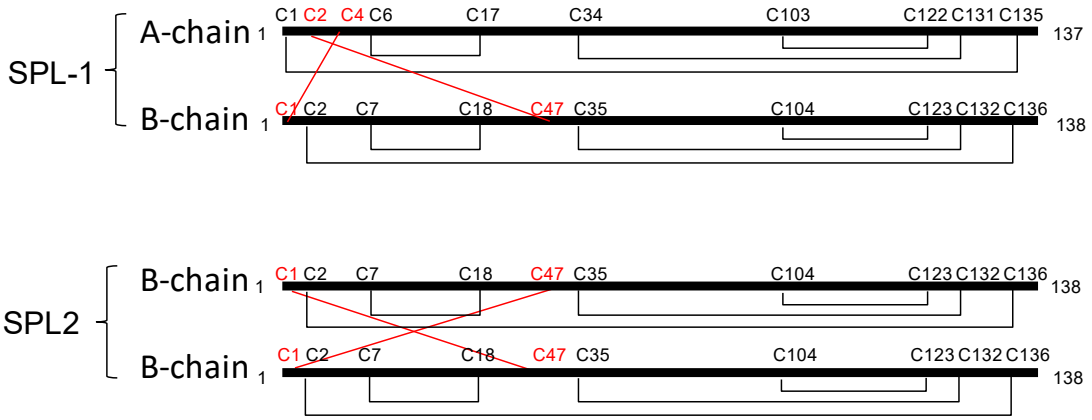

Figure S2. Positions of the interchain disulfide bonds between the constituent chains of SPLs. Thick bars represent constituent polypeptide chains of SPLs. Black thin lines indicate positions of intrachain disulfide bonds, and red lines indicate interchain disulfide bonds. Relative positions of the residues are not proportional.

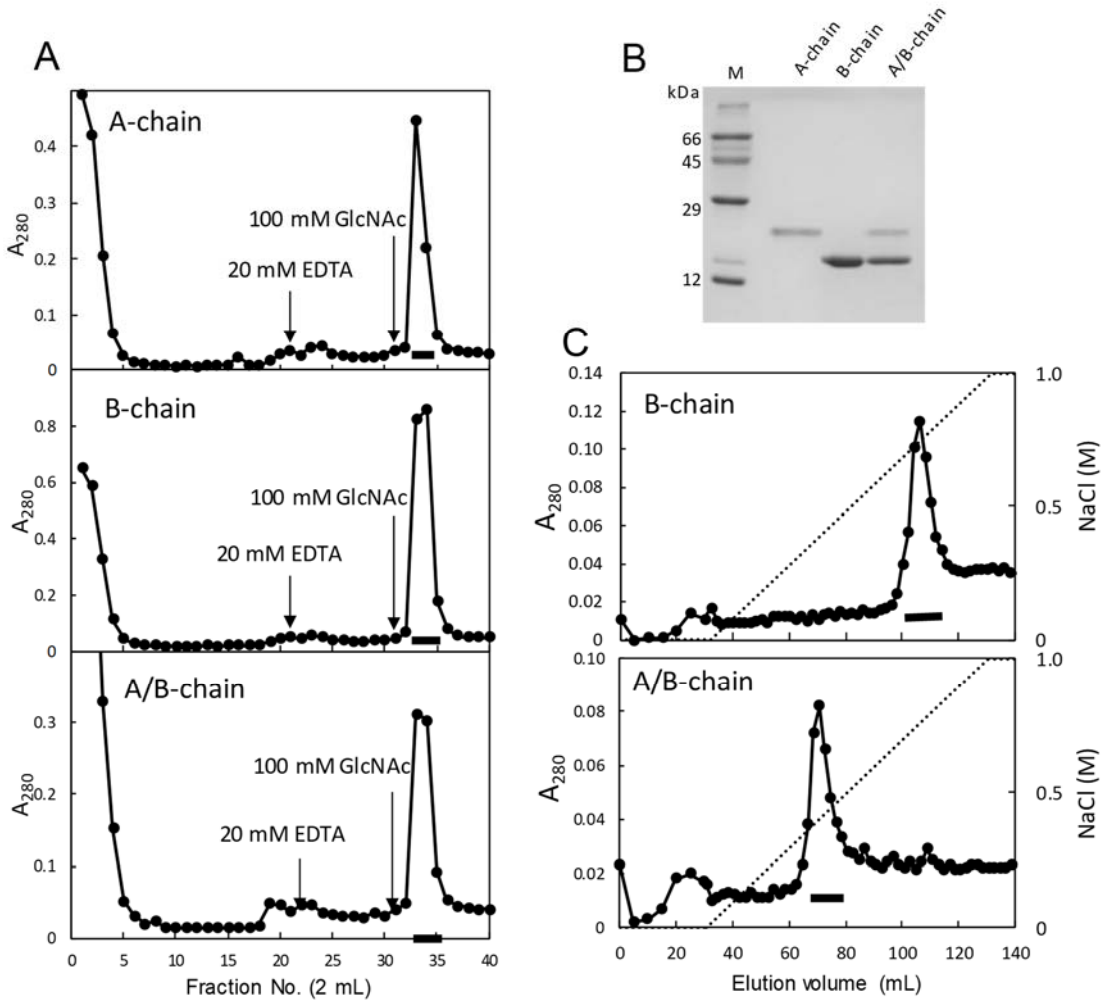

Figure S3. Purification of the recombinant chains of SPLs. A, Affinity chromatography using GlcNAc-Cellufine column. After refolding, A-chain, B-chain, and mixed A- and B-chains were applied to the column and adsorbed proteins were eluted with 20 mM EDTA in TBS, followed by 100 mM GlcNAc in TBS. B, SDS-PAGE of the fractions eluted with GlcNAc. C, Ion-exchange chromatography of the eluate from the affinity chromatography of B-chain and the mixed A- and B-chains.