Binding of Sucrose Octasulphate to the C-Type Lectin-Like Domain of the Recombinant Natural Killer Cell Receptor NKR-P1A Observed by NMR Spectroscopy

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NKR-P1A is a C-type lectin-like receptor on natural killer cells believed to be involved in the cytotoxicity of these cells. Ligands for this protein are not known. Here, we describe the binding of a fully sulphated disaccharide, sucrose octasulphate, by the recombinant C-type lectin-like domain of NKR-P1A. The binding was observed by NMR spectroscopy methods that have recently been described for the screening of compound libraries for bioaffinities, namely the 2D NOESY and saturation transfer difference NMR experiments. ^{1}H titration studies indicate that the binding is specific. These findings raise the possibility that NKR-P1A recognises sulphated natural ligands in common with certain other members of the C-type lectin family.

KEYWORDS:

C-type lectin-like domain · molecular recognition · NMR spectroscopy · oligosaccharides · receptors

Introduction

Natural killer (NK) cells are a class of lymphocytes implicated in cell-mediated surveillance of tumour targets and innate immunity against intracellular bacteria and parasites. NKR-P1A is a homodimeric type-II transmembrane protein of the C-type lectin-like family found on NK cells and NK-like T cells and is believed to be an activator of cytotoxicity.[1] The natural ligands for NKR-P1A that might trigger the cytotoxicity of NK cells are not known.[2a] We previously obtained the C-type lectin-like domain (CTLD) of rat NKR-P1A (MW = 14 kDa) by expression in *Escherichia coli*.[2b] In vitro folding provided a monomeric protein that is well-folded, as assessed by binding experiments with a conformation-sensitive antibody and mass spectrometric data on the disulphide bonds present.[2b] Moreover, the 1D ^{1}H and the 2D ^{1}H,^{15}N HSQC NMR spectra showed large dispersion of the signals, which is characteristic of folded proteins and indicates a considerable amount of β-sheet structure, a feature characteristic for this class of proteins. The availability of a folded protein allows identification of its interactions with small molecules by NMR spectroscopy.

Here we describe the binding of the fully sulphated disaccharide sucrose octasulphate (SOS, Scheme 1) to the recombinant CTLD of NKR-P1A. Binding has been detected by transferred (tr) nuclear Overhauser effects (NOEs) and saturation transfer difference (STD) NMR spectroscopy together with ^{1}H NMR titration studies. The heparin disaccharide IS (Scheme 1) and a pentasaccharide fraction enzymatically generated from heparin were also investigated for their ability to bind to NKR-P1A since sucrose octasulphate has been shown previously to mimic a heparin oligosaccharide when bound by fibroblast growth factor.[3]

Two of these NMR spectroscopy techniques, NOESY and STD, have been applied recently to the screening of libraries of compounds for bioaffinity.[4] In NOESY experiments, strong negative NOEs, so-called tr-NOEs, can be observed between protons when small molecules (molecular weight less than approximately 2 kDa) bind to a receptor protein and undergo sufficiently fast chemical exchange between the bound and free states. The tr-NOEs are readily discriminated from small positive NOEs of small molecules that are not bound. In STD experiments, NMR spectra are recorded with and without saturation of protein resonances to make certain that signals of the small molecule are not directly affected by the saturation pulse. Molecules that are bound are identified from the presence of their signals in the difference spectrum. Moreover, by STD NMR spectroscopy it is

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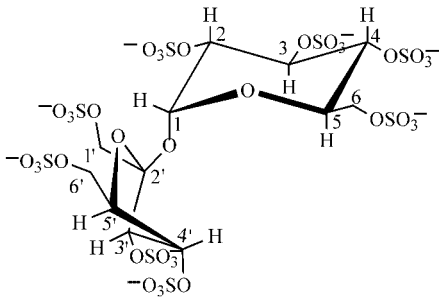
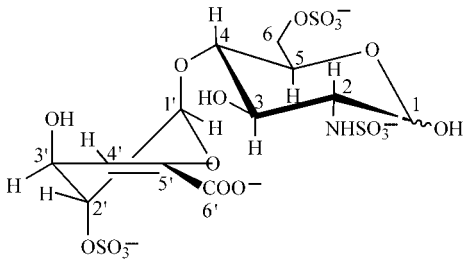


Sucrose octasulphate (SOS)



ΔUA2S(1-4)GlcNS6S (IS)

4-Deoxy-α-L-*threo*-hex-4-ene-pyranosyluronic acid (1-4)-2-amino-2-deoxy-2,6-di-*O*-sulpho-glucopyranose

Scheme 1. *Structures of the NKR-P1A-CTLD-binding molecule sucrose octasulphate and the nonbinding molecule, the heparin disaccharide IS.*

possible to obtain the binding epitope of the ligand.[5] Both methods are applicable for ligands that bind with dissociation constants in the range 10^{-3} – 10^{-7} M.

Results

SOS was subjected to NOESY experiments in the presence and absence of the CTLD of NKR-P1A. In the absence of the protein extremely small NOEs were observed that are of opposite sign to the diagonal (positive NOEs). These NOEs are observed in the trace of H1 of the glucopyranose ring in the NOESY experiment (Figure 1 B). Upon addition of the CTLD of NKR-P1A, the spectrum showed NOEs between H1 and H2 of the glucose ring and across the glycosidic linkage from the glucose H1 atom to H1′a and H1′b of the fructose ring that were of the same sign as the diagonal. These cross peaks arise from SOS that was in the bound state, so-called tr-NOEs (Figure 1 A and C – F). The chemical shift assignments for SOS were taken from the published values.[6] The tr-NOEs for H1 were already observed at a mixing time of 50 ms and increased in intensity with increasing mixing times (Figure 1 C – F). At a mixing time of 500 ms (Figure 1 F), H1 of glucose appeared to show tr-NOEs to all protons

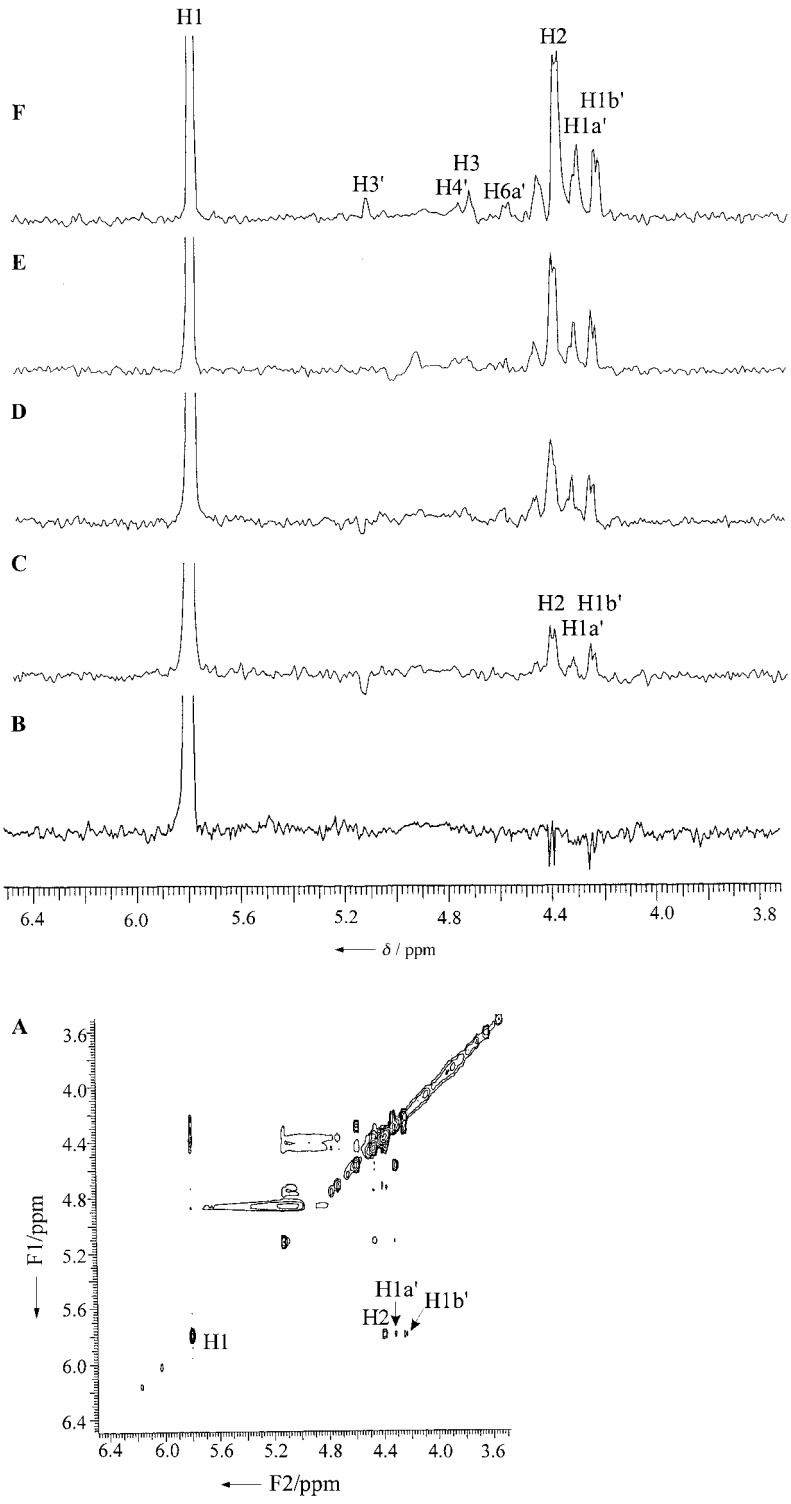


Figure 1. *2D NOESY experiments on SOS with and without the CTLD of NKR-P1A. A) 2D NOESY spectrum of SOS in the presence of the CTLD of NKR-P1A at a mixing time of 100 ms. The molar protein:SOS ratio was 1:10. The labelled cross peaks are of the same sign as the diagonal and therefore arise from tr-NOEs that are caused by the presence of bound SOS. The spectrum was obtained at 600 MHz and a temperature of 15 °C. B – F) F2 traces of a series of 2D NOESY spectra at the ^{1}H chemical shift of glucose H1 ($\delta = 5.82$ ppm) in SOS. The spectrum of SOS without the CTLD of NKR-P1A (B) was recorded at a mixing time of 300 ms. The spectra of SOS in the presence of the CTLD of NKR-P1A were recorded at 50 (C), 100 (D), 300 (E) and 500 ms (F) with a molar protein:SOS ratio of 1:10. The cross signals labelled in (C) are of the same sign as the diagonal because SOS is bound to the protein. Additional cross peaks become visible at longer mixing times (F) and are most likely caused by spin diffusion.*

in the glucose and fructose moiety. This is indicative of spin diffusion transmitted in the protein-bound state of SOS. The heparin disaccharide, IS, (Scheme 1) had positive NOEs (of opposite sign to the diagonal) in the absence of the CTLD of NKR-P1A, and these remained unchanged when the CTLD of NKR-P1A was added (data not shown). This implies that IS does not bind to the CTLD.

^{1}H NMR spectra of the CTLD of NKR-P1A without (Figure 2 A) and with various amounts of SOS (Figure 2 B – H) were recorded in D_2O. The spectrum of the CTLD of NKR-P1A was characteristic of a folded protein (as reported previously[2]). Addition of increasing concentrations of SOS to the protein showed perturbations of several protein resonances. Two of the most prominent changes are indicated in Figure 2 (see arrows). A methyl-group signal at 0.05 ppm increased in intensity when SOS was added. An aromatic resonance at 7.63 ppm was shifted to a lower field and broadened progressively. The results of the titration studies thus support the tr-NOEs data, which indicate that SOS is bound to the CTLD of NKR-P1A. In addition, the results indicate that the binding of SOS involves a specific site on the CTLD of NKR-P1A. A dissociation constant of around 350 $\pm$ 50 μM was estimated for the binding of SOS to the CTLD of NKR-P1A by measuring the integrated area of the well-resolved complex-specific methyl group signal at 0.05 ppm with increasing SOS concentrations.

1D STD NMR experiments were performed for SOS in the presence of the CTLD of NKR-P1A. Figure 3 A shows the 1D ^{1}H NMR spectrum of SOS with off-resonance irradiation at $\delta = -4.8$ ppm. Figure 3 B shows the STD spectrum (spectrum A minus the spectrum with on-resonance irradiation at $\delta =$ 0.9 ppm). Here, all ^{1}H signals of SOS are visible. In agreement with the titration studies and tr-NOEs, the STD spectrum corroborates the conclusion that SOS is bound to the CTLD of NKR-P1A.

A control STD experiment was performed by using albumin and SOS (Figure 3 C and D). Albumin has a similar isoelectric point (pI value) to that of the recombinant CTLD of NKR-P1A (albumin, pI = 5.1; CTLD of NKR-P1A, pI = 5.9[2b]). The STD spectrum here did not show any oligosaccharide signals, in contrast to Figure 3 B, and thus SOS appears not to bind to albumin. We have further investigated the possibility that heparin may be a ligand of NKR-P1A by carrying out STD experiments with either the IS heparin disaccharide (Figure 3 E and F) or a heparin pentasaccharide fraction (Figure 3 G and H) in which one component contains the same number of sulphates as SOS (see the Experimental Section), in the presence of NKR-P1A. No oligosaccharide peaks were visible in the STD spectra, which shows that neither the disaccharide nor the pentasaccharide binds to the CTLD of NKR-P1A. This is in agreement with ELISA-type experiments, which showed no binding of the polysaccharide heparin to the immobilised CTLD of NKR-P1A (unpublished observations).

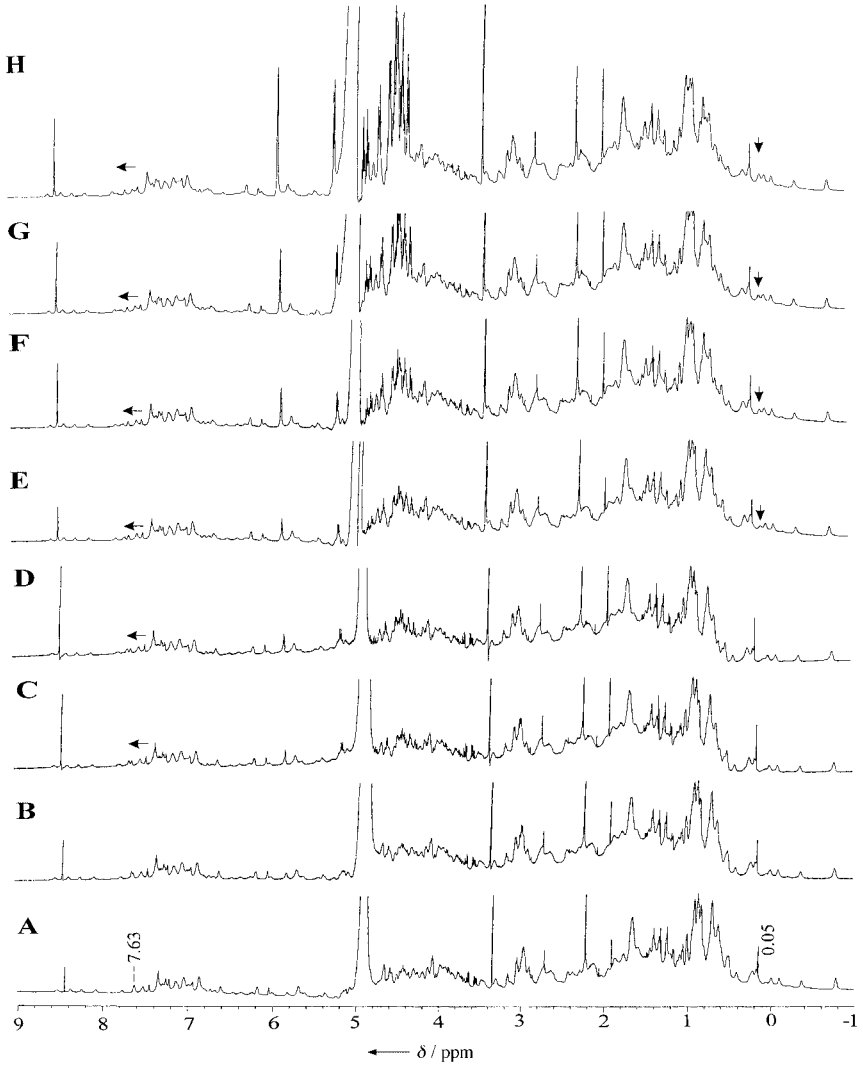


Figure 2. 1D ^{1}H NMR spectra of the CTLD of NKR-P1A alone (60 μM; A) and in the presence of SOS (40 μM, (B); 100 μM, (C); 130 μM, (D); 160 μM, (E); 240 μM, (F); 360 μM, (G); 600 μM, (H)). Spectra A and E – H were recorded at 600 MHz and spectra B – D at 500 MHz. The temperature used was 15 °C. The signals of the CTLD of NKR-P1A at $\delta = 0.05$ ppm and $\delta = 7.63$ ppm, which are affected by addition of SOS, are indicated by arrows.

Discussion

1D ^{1}H NMR spectroscopy titration experiments, 2D tr-NOESY and STD NMR spectra show that the fully sulphated disaccharide SOS binds to the CTLD of NKR-P1A and that the binding is specific. No evidence was found for binding to the disaccharide unit of heparin IS or to a heparin pentasaccharide fraction generated enzymatically from heparin. It will be of interest to investigate the binding of other naturally occurring sulphated glycosaminoglycan sequences to the CTLD of NKR-P1A, particularly as an affinity for sulphated carbohydrates has been noted previously for various members of the C-type lectin-like

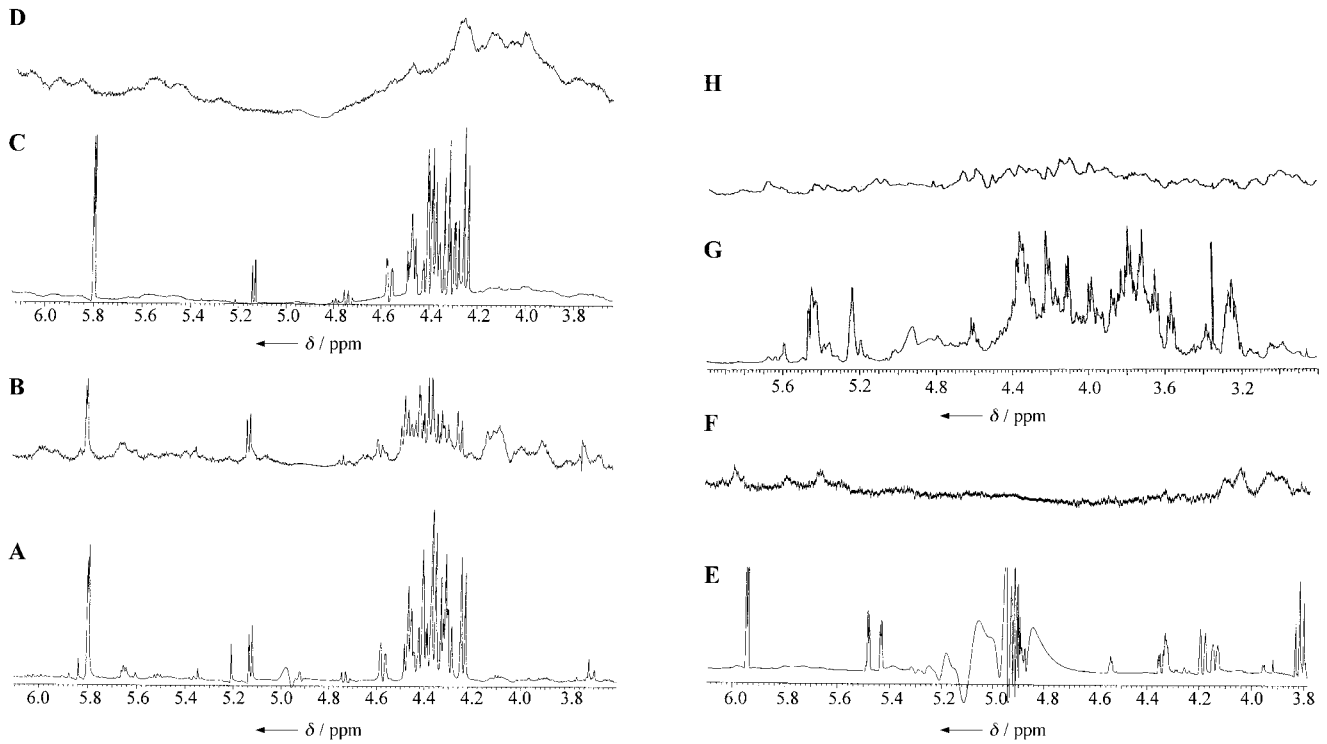


Figure 3. *STD NMR spectra used to identify molecules bound by the CTLD of NKR-P1A. A) 1D ^{1}H NMR spectrum of SOS and the CTLD of NKR-P1A recorded with off-resonance irradiation at $\delta = -4.8$ ppm and (B) the corresponding STD spectrum (spectrum A minus the spectrum with on-resonance irradiation at $\delta = 0.9$ ppm). C) 1D ^{1}H NMR spectrum of SOS and albumin recorded with off-resonance irradiation at $\delta = -4.8$ ppm and (D) the corresponding STD spectrum (spectrum C minus the spectrum with on-resonance irradiation at $\delta = 0.6$ ppm). E) 1D ^{1}H NMR spectrum of the heparin disaccharide, IS, and the CTLD of NKR-P1A recorded with off-resonance irradiation at $\delta = -4.8$ ppm and (F) the corresponding STD spectrum (spectrum E minus the spectrum with on-resonance irradiation at $\delta = 0.9$ ppm). The molar ratio of protein to IS or SOS was 1:30. G) 1D ^{1}H NMR spectrum of the heparin pentasaccharide fraction, generated enzymatically from heparin (see the Experimental Section), and the CTLD of NKR-P1A recorded with off resonance irradiation at $\delta = -4.8$ ppm and (H) the corresponding STD spectrum (spectrum (G) minus the spectrum with on resonance irradiation at $\delta = 0.5$ ppm). The molar ratio of protein to heparin pentasaccharide fraction was 1:25.*

family. The inhibitory C-type lectin-like receptors of NK cells, Ly49A and Ly49C, bind to major histocompatibility class (MHC) I molecules,[7] but they have been found to bind to sulphated polysaccharides as well.[8] The X-ray structure of the MHC-class-I/Ly49A complex has been reported.[9] The recombinant molecule, H-2D^d, used in the crystallisation experiment was nonglycosylated but it has been suggested that the carbohydrate chain on the natural MHC class I molecule could also be bound by Ly49A.[10] An affinity for sulphate groups has been reported for other proteins of the C-type superfamily. The leukocyte-to-endothelium adhesion molecules, E- and L-selectins, are C-type lectins that bind sulphated as well as sialyl oligosaccharides of the Lewis^a and Lewis^x series.[11] The third member of the selectin family, P-selectin, requires the presence of sulphotyrosines in addition to a sialyl-Lewis^x-type oligosaccharide for high affinity binding to its counter-receptor PSGL-1.[12] Direct binding of P-selectin to a sulphotyrosine has been demonstrated by using a synthetic ligand analogue in the clustered state and a synergism observed between sialyl-Lewis^x and sulphotyrosine.[13] The interactions with the two ligand classes have been revealed in atomic detail for P-selectin in complex with the N-terminal domain of PSGL-1, which contains both sialyl-oligosaccharide and sulphotyrosine ligands.[14] In a recently produced X-ray structure of the eosinophilic major basic protein (EMBP),[15] which is a member of the C-type lectin-like superfamily, several sulphated molecules (components of the cryoprotectant) were bound. The binding of the sulphate groups might point to a natural sulphated carbohydrate ligand, and this is supported by the finding that heparin reverses some of the functions of EMBP. Availability of 3D structures of sulphate/protein complexes allows comparisons between mechanisms of sulphate binding. It has been noted[16] that P-selectin and EMBP employ one arginine residue that is located in a similar position in both molecules in their interaction with sulphate. Comparison of the primary amino acid sequence of NKR-P1A with P-selectin reveals two serine residues in the CTLDs that are in homologous positions, Ser_{149}, Ser_{150}[14] and Ser_{46}, Ser_{47},[17] respectively. In P-selectin these residues are involved in binding a sulphate group of the counter-receptor.[14] Further similarities are not apparent.

A common theme has emerged in the topology of ligand binding regions for C-type lectin and lectin-like proteins, whether they interact with oligosaccharide or polypeptide or both oligosaccharide and polypeptide ligands.[16] In all cases structurally investigated so far, binding regions have been in the upper part of the fold that is opposite the N and C termini. In addition, the binding involves a limited region that is broadly equivalent to the mannose binding site of the prototype of C-type lectins, the mannose-binding protein MBP-A.[18] Identification of the binding site of SOS on NKR-P1A might thus provide a clue to a natural ligand-binding region.

Experimental Section

Materials and methods: Sucrose octasulphate potassium salt (MW = 1287.55 Da) was from Toronto Research Chemicals, Inc. The sodium salt of the heparin disaccharide IS (MW = 665.4 Da) and albumin (chicken egg) were from Sigma-Aldrich. The heparin pentasaccharide fraction was prepared by oxymercuration treatment[19a] of hexasaccharide fragments derived from heparin lyase I digestion[19b] of porcine mucosa heparin (Sigma), and fractionation by gel filtration. The cleaved nonreducing terminal unsaturated hexuronic acid residue (ΔUA) and the mercuric reagent were removed by gel filtration on a short Sephadex G10 column. The resulting pentasaccharide fraction was analysed by electrospray mass spectrometry[19b] and found to contain components with seven (~37%), six (~28%), eight (~24%) and five sulfate groups (~13%). The fraction was quantified by carbazole assay[19c] with D-glucurono-6,3-lactone as the standard.

The CTLD of NKR-P1A (14 kDa) was obtained by expression in *E. coli*, in vitro folding, cleavage of the vector-derived N-terminal tag and purification as described previously.[2b] After reversed-phase HPLC purification and lyophilisation, the CTLD was dissolved in phosphate buffer (10 mM), ethylenediaminetetraacetate (EDTA; 0.02 mM) and sodium azide (0.02%) in D_2O (550 μL), lyophilised one more time and finally D_2O (550 μL) was added. The protein concentration was determined by densitometry of Coomassie-blue-stained SDS-PAGE with soybean trypsin inhibitor as a standard.

NMR spectroscopy: The NMR experiments were carried out on Varian UNITY INOVA and UNITYplus spectrometers at proton frequencies of 600 and 500 MHz, respectively. The spectra were recorded at 15 °C in D_2O (550 μL) at pH* 7.0 (pH* values are pH meter readings uncorrected for deuterium isotope effects). Spectra were referenced to d_4-trimethylsilylpropionic acid (Sigma-Aldrich).

1D ^{1}H NMR titration studies: A series of 1D ^{1}H NMR spectra were recorded on a sample of the CTLD of NKR-P1A (60 μM) with various concentrations of added SOS (0–600 μM). The disaccharide was added from a concentrated stock solution to minimise dilution effects and the pH value was re-adjusted to 7.0 where necessary. Each spectrum was recorded with a spectral width of 7509 Hz and 5696 scans. The spectra were processed without a window function except for the protein-only spectrum, where a gaussian window function was applied prior to Fourier transformation. The H_2O signal was suppressed by low-power presaturation during the relaxation delay.

The dissociation constant K_d for the binding of SOS to NKR-P1A was obtained by using the equations for a simple bimolecular binding process[20] [Eqs. (1) and (2)], where [E] and [L] are the concentrations of the free CTLD of NKR-P1A and free SOS in solution, respectively, [EL] is the concentration of the complex, $[E_T]$ and $[L_T]$ are the total concentrations of the protein and ligand in the sample and the normalised area of the complex-specific signal is equal to $[EL]/[E_T]$.

$$K_d = \frac{[E][L]}{[EL]} \quad (1)$$

$$[EL] = \frac{1}{2}\{[E_T] + [L_T] + K_d - (([E_T] + [L_T] + K_d)^2 - 4[E_T][L_T])^{1/2}\} \quad (2)$$

2D NOESY and transferred NOESY: A 2D NOESY spectrum was recorded on SOS (600 μM) in phosphate buffer (10 mM), EDTA (0.02 mM) and sodium azide (0.02%) in D_2O at pH* 7.0 with a mixing time of 300 ms. A series of 2D NOESY spectra were recorded on a mixture of the CTLD of NKR-P1A (60 μM) and sucrose octasulphate (600 μM). Mixing times were 50, 100, 300 and 500 ms. All spectra were recorded with spectral widths of 7500 Hz and 32 scans. A total of 180 (t_1) × 4000 (t_2) data points were recorded for each experiment. Prior to Fourier transformation, the data matrix was multiplied with a gaussian function. The H_2O signal was suppressed by low-power presaturation during the relaxation time. The relaxation delay was 2.5 s. The residual H_2O signal was removed during data processing.

1D ^{1}H saturation difference NMR spectroscopy: 1D STD NMR experiments[4b, 5c] were recorded interleaved with 4 scans. The H_2O resonance was suppressed by the pulse gradient echo Watergate method[21] in experiments with the disaccharide SOS and the heparin disaccharide IS, whereas experiments with the heparin pentasaccharide fraction employed presaturation during the relaxation delay to suppress the H_2O signal. The spectra with off-resonance irradiation were at $\delta = -4.8$ ppm. Saturation was achieved by using 120 selective hyperbolic secant pulses of 25 ms (pulse bandwidth 1.6 ppm), each separated by a 1-ms delay to saturate the protein, and a total saturation time of 3.0 s. The relaxation delay was set to 500 ms.

The STD spectrum of albumin (60 μM) and SOS (1.8 mM) was recorded with a sweep width of 1500 Hz and 5824 scans in phosphate buffer (10 mM), EDTA (0.02 mM) and sodium azide (0.02%) in D_2O at pH* 7.0. The STD spectrum was obtained from the off-resonance spectrum minus the corresponding spectrum with on-resonance at $\delta =$ 0.9 ppm.

The STD spectrum of the CTLD of NKR-P1A (60 μM) and SOS (1.8 mM) was recorded with a sweep width of 1500 Hz and 5824 scans in the presence of NaCl (150 mM). The STD spectrum was obtained from the off-resonance spectrum minus the corresponding spectrum with on-resonance irradiation at $\delta = 0.9$ ppm. An STD spectrum was obtained of the CTLD of NKR-P1A in the presence of SOS as before, with the addition of urea (5.2 M) and dithiothreitol (5 mM). This spectrum did not show any signals from SOS (data not shown).

The STD spectrum of the CTLD of NKR-P1A (60 μM) and the heparin disaccharide IS (1.8 mM) was recorded with a sweep width of 7500 Hz and 4480 scans in phosphate buffer (10 mM), EDTA (0.02 mM) and sodium azide (0.02%) in D_2O at pH* 7.0. The STD spectrum was obtained from the off-resonance spectrum minus the corresponding spectrum with on-resonance irradiation at $\delta = 0.6$ ppm.

The STD spectrum of the CTLD of NKR-P1A (60 μM) and the heparin pentasaccharide fraction (1.5 mM) was recorded with a sweep width of 8500 Hz and 4480 scans in phosphate buffer (10 mM), EDTA (0.02 mM) and sodium azide (0.02%) in D_2O at pH* 7.0. The STD spectrum was obtained from the off-resonance spectrum minus the corresponding spectrum with on-resonance irradiation at $\delta =$ 0.5 ppm.

The following control experiments were performed: 1) Samples containing only SOS, IS and the heparin pentasaccharide fraction were subjected to STD experiments under saturation conditions identical to those in the presence of protein. The STD spectra did not contain any signals. 2) Spectra with off-resonance irradiation of the CTLD of NKR-P1A in the presence of SOS, IS or the heparin pentasaccharide fraction and of albumin in the presence of SOS were recorded. Subtraction of these two spectra did not reveal any signals.

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[1] J. C. Ryan, W. E. Seaman, *Immunol. Rev.* **1997**, *155*, 79 – 89.
[2] a) H. Kogelberg, E. Montero, S. Bay, A. M. Lawson, T. Feizi, *J. Biol. Chem.* **1999**, *274*, 30 335 – 30 336; b) H. Kogelberg, A. M. Lawson, F. W. Muskett, R. A. Carruthers, T. Feizi, *Protein Expression Purif.* **2000**, *20*, 10 – 20.
[3] D. B. Volkin, A. M. Verticelli, K. E. Marfia, C. J. Burke, H. Mach, C. R. Middaugh, *Biochim. Biophys. Acta* **1993**, *1203*, 18 – 26.
[4] a) B. Meyer, T. Weimar, T. Peters, *Eur. J. Biochem.* **1997**, *246*, 705 – 709; b) M. Mayer, B. Meyer, *Angew. Chem.* **1999**, *111*, 1902 – 1906; *Angew. Chem. Int. Ed.* **1999**, *38*, 1784 – 1788.
[5] a) H. Maaheimo, P. Kosma, L. Brade, H. Brade, T. Peters, *Biochemistry* **2000**, *39*, 12 778 – 12 788; b) M. Vogtherr, T. Peters, *J. Am. Chem. Soc.* **2000**, *122*, 6093 – 6099; c) M. Mayer, B. Meyer, *J. Am. Chem. Soc.* **2001**, *123*, 6108 – 6117.
[6] a) U. R. Desai, I. R. Vlahov, A. Pervin, R. J. Linhardt, *Carbohydr. Res.* **1995**, *275*, 391 – 401; b) J. Shen, L. E. Lerner, *Carbohydr. Res.* **1995**, *273*, 115 – 127.
[7] F. Takei, J. Brennan, D. L. Mager, *Immunol. Rev.* **1997**, *155*, 67 – 77.
[8] B. F. Daniels, M. C. Nakamura, S. D. Rosen, W. M. Yokoyama, W. E. Seaman, *Immunity* **1994**, *1*, 785 – 792.
[9] J. Tormo, K. Natarajan, D. H. Margulies, R. A. Mariuzza, *Nature* **1999**, *402*, 623 – 631.
[10] P. Parham, *Curr. Biol.* **2000**, *10*, R195 – R197.
[11] a) C.-T. Yuen, A. M. Lawson, W. Chai, M. Larkin, M. S. Stoll, A. C. Stuart, F. X. Sullivan, T. J. Ahern, T. Feizi, *Biochemistry* **1992**, *31*, 9126 – 9131; b) S. D. Rosen, *Histochemistry* **1993**, *100*, 185 – 191.
[12] a) T. Pouyani, B. Seed, *Cell* **1995**, *83*, 333 – 343; b) D. Sako, K. M. Comess, K. M. Barone, R. T. Camphausen, D. A. Cumming, G. D. Shaw, *Cell* **1995**, *83*, 323 – 331; c) P. P. Wilkins, K. L. Moore, R. P. McEver, R. D. Cummings, *J. Biol. Chem.* **1995**, *270*, 22 677 – 22 680.
[13] C. Galustian, R. A. Childs, M. S. Stoll, H. Ishida, M. Kiso, T. Feizi, *Immunology* **2002**, *105*, 350 – 359.
[14] W. S. Somers, J. Tang, G. D. Shaw, R. T. Camphausen, *Cell* **2000**, *103*, 467 – 479.
[15] G. J. Swaminathan, A. J. Weaver, D. A. Loegering, J. L. Checkel, D. D. Leonidas, G. J. Gleich, K. R. Acharya, *J. Biol. Chem.* **2001**, *276*, 26 197 – 26 203.
[16] H. Kogelberg, T. Feizi, *Curr. Opin. Struct. Biol.* **2001**, *11*, 635 – 643.
[17] R. Giorda, W. A. Rudert, C. Vavassori, W. H. Chambers, J. C. Hiserodt, M. Trucco, *Science* **1990**, *249*, 1298 – 1300.
[18] W. I. Weis, K. Drickamer, W. A. Hendrickson, *Nature* **1992**, *360*, 127 – 134.
[19] a) W. Chai, H. Kogelberg, A. M. Lawson, *Anal. Biochem.* **1996**, *237*, 88 – 102; b) W. Chai, J. Luo, C. K. Lim, A. M. Lawson, *Anal. Chem.* **1998**, *70*, 2060 – 2066; c) T. Bitter, H. M. Muir, *Anal. Biochem.* **1962**, *4*, 330 – 334.
[20] J. Feeney, B. Birdsall in *NMR of Macromolecules A Practical Approach*, 2nd ed. (Ed.: G. C. K. Roberts), Oirl Press at Oxford University Press, Oxford, in press.
[21] G. Wider, K. Wüthrich, *J. Magn. Reson. Ser. B* **1993**, *102*, 239 – 241.

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