Selective Binding of the Scavenger Receptor C-type Lectin to Lewis\textsuperscript{x} Trisaccharide and Related Glycan Ligands*  

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The scavenger receptor C-type lectin (SRCL)\textsuperscript{1} is an endothelial receptor that is similar in organization to type A scavenger receptors for modified low density lipoproteins but contains a C-type carbohydrate-recognition domain (CRD). Fragments of the receptor consisting of the entire extracellular domain and the CRD have been expressed and characterized. The extracellular domain is a trimer held together by collagen-like and coiled-coil domains adjacent to the CRD. The amino acid sequence of the CRD is very similar to the CRD of the asialoglycoprotein receptor and other galactose-specific receptors, but SRCL binds selectively to asialo-ornosomucoid rather than generally to asialoglycoproteins. Screening of a glycan array and further quantitative binding studies indicate that this selectivity results from high affinity binding to glycans bearing the Lewis\textsuperscript{x} trisaccharide. Thus, SRCL shares with the dendritic cell receptor DC-SIGN the ability to bind the Lewis\textsuperscript{x} epitope. However, it does so in a fundamentally different way, making a primary binding interaction with the galactose moiety of the glycan rather than the fucose residue. SRCL shares with the asialoglycoprotein receptor the ability to mediate endocytosis and degradation of glycoprotein ligands. These studies suggest that SRCL might be involved in selective clearance of specific desialylated glycoproteins from circulation and/or interaction of cells bearing Lewis\textsuperscript{x}-type structures with the vascular endothelium.

The scavenger receptor C-type lectin (SRCL)\textsuperscript{2} is also known as collectin-placenta 1 (CL-P1). Although originally cloned from liver and placenta, SRCL is expressed in many tissues (1, 2). The receptor is found in endothelial cells from human umbilical vein and artery as well as in vascular endothelial cells of the heart, suggesting that it may be widely distributed in endothelia. The SRCL polypeptide contains an N-terminal intracellular domain, transmembrane anchor, and the extended extracellular domain (Fig. 1). The extracellular portion of the receptor is composed of a neck that contains coiled-coil and collagen-like regions and a C-terminal carbohydrate-recognition domain (CRD).

The binding properties of SRCL have only been partially characterized. Binding to oxidized low density lipoprotein particles has been demonstrated and, by analogy to the type A scavenger receptors, this activity has been attributed to the collagen-like domain (1). Binding to Gram-negative bacteria has also been associated with the collagen-like domain, because a variant form of the protein lacking CRDs still shows binding activity for bacteria when expressed in Chinese hamster ovary cells (2). Although the cytoplasmic domain of SRCL contains a potential endocytosis signal motif, conflicting data have been presented regarding the uptake of particulate ligands into cells (1, 2).

C-type CRDs fall broadly into two subgroups, those that bind galactose and related ligands and those that bind mannose, N-acetylglucosamine, and fucose. Mutagenesis of galactose-binding C-type CRDs, combined with studies in which the specificity of a mannose-binding CRD was converted to galactose binding, have led to the identification of a small set of residues that are essential for high affinity binding to galactose (3, 4). Examination of the sequence of SRCL reveals that all of these key residues are present.\textsuperscript{2} Thus, SRCL differs from DC-SIGN, the macrophage mannose receptor, serum mannose-binding protein, and other C-type lectins known to have roles in pathogen recognition, all of which fall into the second subgroup. Semiquantitative binding studies using cells expressing SRCL suggest that the receptor does bind sugar-containing ligands, with some preference for N-acetylgalactosamine (5).

The overall organization of SRCL shares features of both the type A scavenger receptors for modified low density lipoprotein and the collectins, which are soluble C-type lectins involved in pathogen recognition. The arrangement of domains in SRCL is most like the arrangement of the type A scavenger receptors, with the CRDs replacing cysteine-rich domains that are not related in sequence (6). Sequence comparisons reveal sequence similarities between the collagen-like and coiled-coil domains of SRCL and all three of the type A scavenger receptors, with particularly strong similarity to SR-A 3 (Fig. 1) (7). Although the collagen-like and coiled-coil domains would have similar elongated conformations in SRCL and the collectins, sequence comparisons provide no evidence for homology between SRCL and the collectins, and the domains are actually arranged in reverse order in the polypeptides. In addition, comparison of the CRD of SRCL with other C-type lectins shows that, in evolutionary terms, this domain falls within the family of type II transmembrane receptors that includes the asialoglycoprotein receptor, rather than forming part of the collectin cluster. Thus, it seems likely that the presence of similar domains in

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1 The abbreviations used are: SRCL, scavenger receptor C-type lectin; SR, scavenger receptor; CRD, carbohydrate-recognition domain; BSA, bovine serum albumin; LNFP, lacto-N-fucopentaose.

2 See www.imperial.ac.uk/research/animallectins for sequence alignments.
SRCL and the collectins represents independent gene shuffling events that have converged on a similar set of protein structural elements.

To assess the biological functions of SRCL, both the sugar-binding characteristics and intracellular trafficking properties of the receptor need to be better defined. To this end, the results of this study demonstrated that the CRD of SRCL binds with unusually high selectivity to glycans containing the Lewis$^*$ epitope, that the CRDs are clustered in a trimer, and that the receptor is able to direct uptake and degradation of glycoprotein ligands.

**EXPERIMENTAL PROCEDURES**

**Materials—**Oligosaccharides and LNFP III-BSA were purchased from Dextra Ltd. (Reading, UK). Galactose-BSA was obtained from E-Y Laboratories (San Mateo, CA). Radiosotopes were from Amersham Biosciences. Rabbit polyclonal antibodies to the CRD from SRCL were generated by Eurogentec (Seraing, Belgium) following their standard protocol using 2 mg of bacterially expressed protein as antigen.

Cloning of SRCL cDNA—cDNA encoding human SRCL was amplified from human placental cDNA (Clontech) in two sections using the following primers: N-terminal section forward, 5′-AACCCACGCGTCACCCCAATGAAAGAAGCCCATGCGCTC-3′; N-terminal section reverse, 5′-TTGAGCTGACCATGCTTGGAGTCTACTAGCTTC-3′; C-terminal section forward, 5′-AAGTGGTCATCATGAACCTCAACAATG-3′; C-terminal section reverse, 5′-TTGCCGCCGCTGTTATAAGCATGAGTCTGGTCTG-3′. PCR products were digested with the vector pCRII-TOPO using the TOPO cloning kit (Invitrogen) and sequenced using an Applied Biosystems Prism 310 genetic analyzer. Segments of the cDNAs that were free of reverse transcription errors were cloned using convenient restriction sites.

Expression of the CRD from SRCL in Bacteria—The region of the cDNA coding for the CRD only (residue 603 to the C terminus) was amplified using the above unique Clontech primers with 5′-AACCCACGCGTCACCCCAATGAAAGAAGCCCATGCGCTC-3′; reverse primer, 5′-TTGCCGCCGCTGTTATAAGCATGAGTCTGGTCTG-3′. PCR products were digested at the FseI and NotI sites downstream of the PCR products were digested at the FseI and NotI sites in the primers and FseI, followed by trimming of the 3′ end (8). The correct reading frame was generated by digesting the vector with FseI,ompC packaging cells to produce a pseudovirus that was used to infect Rat-6 fibroblasts, and fibroblast lines stably expressing SRCL were selected using G418 (17). Cells were harvested by scraping, suspended in ~10 volumes of 125 mM NaCl, 10 mM Tris-Cl, pH 7.5, and 0.5% Triton X-100, sonicated briefly, incubated 1 h at 4 °C, and spun for 2 min at 18,000 × g. The supernatant was analyzed by SDS-polyacrylamide gel electrophoresis on a 12% gel, which was blotted into nitrocellulose and probed with antibodies raised against the CRD (18).

**Analytical Ultracentrifugation—**Equilibrium sedimentation analysis was carried out in a Beckman Optima XL-A analytical Ultracentrifuge equipped with absorbance optics using an An60Ti rotor at 20 °C. Proteins were dialyzed extensively against 10 mM NaCl, pH 7.8, 0.1 M NaCl, pH 7.5, and 0.1 M NaCl. The extracellular domain of SRCL was analyzed at 5°C, rotor speeds of 10,000 and 21,000 rpm were used. Equilibrium distributions from three different loading concentrations (0.5, 0.25, and 0.125 mg/ml for the extracellular domain and 0.2, 0.1, and 0.05 mg/ml for the CRD) were analyzed simultaneously using the Nonlin curve fit program supplied with the instrument. Partial specific volumes for the SRCL fragments were determined from their amino acid composition and estimates of the extent of glycosylation (19).

**Labeling of the Extracellular Domain of SRCL—**For radio-iodination, the extracellular domain of SRCL, ~100 μg of protein in 1.5 ml, was dialyzed against 150 mM NaCl, 25 mM HEPES, pH 7.8, 5 mM CaCl$_2$ and reacted with 50 μCi of $^{125}$I-Bolton-Hunter reagent for 15 min at room temperature. Labeled protein was repurified by affinity chromatography on CL-Sepharose. For fluorescent labeling, 1 μl of protein was dialyzed against water, lyophilized, dissolved in 0.5 ml of 0.5 M NaCl, 20 mM Na-bicine, 5 mM CaCl$_2$ and reacted with 75 μg of fluorescein isothiocyanate that was dissolved in 1 mg/ml in dimethyl sulfoxide and added as five aliquots of 1 μl, followed by reaction overnight at 4 °C. EDTA was added to a concentration of 10 mM, and the labeled protein was repurified by gel filtration on a 10 × 300-mm Superdex 200 column (Amersham Biosciences) eluted with 0.1 M NaCl, 0.1 mM Tris-Cl, pH 7.8, 2.5 mM EDTA at a flow rate of 0.5 ml/min.

**Probing of Ligands—**Glycoproteins were resolved on a 17.5% SDS-polyacrylamide gel and transferred to nitrocellulose. All of the glycoproteins were of bovine origin, except orosomucoid, which was from human serum. The blot was blocked at room temperature for 1 h with 2% (w/v) BSA in Tris-buffer (25 mM NaCl, 25 mM Tris-Cl, pH 7.8, and 25 mM CaCl$_2$) and reacted for 1 h with the radio-iodinated extracellular domain of SRCL in the same solution at a concentration of ~1 μg/ml. The blot was washed four times for 5 min each with cold wash buffer, and radioactivity was detected using a phosphorimaging device from Molecular Dynamics. Neoglycolipids, prepared by coupling digos- saccharides to phosphatidylethanolamine dipalmitate, were resolved by two-dimensional chromatography and fixed with isobutylmethylacrylate (21). The chromatograms were then blocked and probed as for the glycoprotein blots. Fluorescein-labeled extracellular domain was used to probe the glycan array following the standard procedure of Core H of the Consortium for Functional Glycomics. 

www.functionalglycomics.org
**Solid Phase Binding Assays**—Polystyrene wells were coated overnight with either CRD or the extracellular domain of SRCL at a concentration of ~50 μg/ml in loading buffer. Neoglycoprotein reporter ligands were created by iodination (22). Binding competition experiments and determination of the pH dependence of binding were performed as previously described (17). Binding data were fitted using SigmaPlot software (Jandel Scientific).

**Molecular Modeling**—The crystal structure of the galactose-binding derivative of mannose-binding protein with bound galactose was used as a starting point (23). The structure of the Lewisx trisaccharide abstracted from the crystal structures of this ligand bound to DC-SIGN was used to model the ligand (24), although similar results were obtained using coordinates derived from the crystal structure of sialyl-Lewisx bound to E-selectin and from the NMR structure of the free oligosaccharide. The galactose moiety of Lewisx was manually superimposed onto the galactose in the mannose-binding protein structure using Insight II modeling software.

**Endocytosis Experiments**—Analysis of uptake and degradation of 125I-Gal-BSA or 125I-LNFP III-BSA by fibroblasts expressing SRCL was performed as described previously (25).

### RESULTS

**Expression and Characterization of the SRCL Extracellular Domains**—To initiate a study of the ligand-binding properties of SRCL, the C-terminal CRD was expressed in bacteria, whereas the entire extracellular domain was expressed in Chinese hamster ovary cells (Fig. 1). In the latter case, the insulin signal sequence was used to direct the protein to the endoplasmic reticulum so that it could be modified by hydroxylation and glycosylation before secretion into the medium. Both fragments of SRCL could be purified by affinity chromatography on galactose-Sepharose (Fig. 2). The CRD, on its own, binds weakly and washes off the affinity column even in the presence of Ca2+. The extracellular domain binds more effectively, and columns of sufficient capacity can be washed with Ca2+-containing buffer and eluted with EDTA. The extracellular domain fragment migrated as a broad band on SDS-polyacrylamide gels, suggesting that there is considerable heterogeneity in post-translational modification. The apparent molecular weight of 110–115 kDa deduced from the gels was consistent with the predicted presence of up to 13 N-linked glycans, 8 O-linked glycans, and mono- or disaccharide residues attached to hydroxylysine residues, all appended to a core polypeptide of 75 kDa.

Weak binding to highly substituted sugar resins is often characteristic of monomeric forms of C-type lectins. Therefore, the oligomeric states of the fragments were analyzed by equilibrium analytical ultracentrifugation (Fig. 3). The results demonstrated that the CRD is monomeric, but the entire extracellular domain formed a stable trimer. This result is consistent with the presence of the collagen-like domain, which would be expected to direct trimerization. In addition, the sequence of the N-terminal portion of the extracellular domain was predicted to form a 3-stranded coiled coil of α-helices, which would further stabilize the trimer. The relatively weak binding of both the CRD and the extracellular domain to galactose-Sepharose contrasts with other galactose-binding C-type lectins, such as the asialoglycoprotein receptor and the Kupffer cell receptor. Both monomeric and trimeric fragments of these receptors bind tightly to this resin and can only be eluted with EDTA, indicating that these receptors have inherently higher affinity for galactose. These results suggest that galactose on its own is not an optimal ligand for SRCL.

**Binding of SRCL to Lewisx and Related Structures**—An initial screening for higher affinity ligands was undertaken by testing the binding of SRCL to a selection of glycoproteins. The glycoproteins were resolved by SDS-polyacrylamide gel electrophoresis, blotted onto nitrocellulose, and probed with the radiolabeled extracellular domain of SRCL (Fig. 4). Strong binding to asialo-orosomucoid is evident, but no binding to other desialylated glycoproteins was detected. Binding to glycoproteins bearing sialylated complex, high mannose, or sulfated N-linked glycans was also not detected.

There are two relatively unusual features to the N-linked
glycans on asialo-orosomucoid. First, many of the glycans have highly branched tri- and tetra-antennary structures. Second, many of these branches bear outer-arm fucose residues (26). The contribution of these two features to the binding of SRCL to asialo-orosomucoid was assessed by testing the binding to highly branched glycans lacking fucose residues and glycans bearing single terminal fucose residues. The glycans were presented as neoglycolipids resolved by thin layer chromatography (Fig. 5). The radiolabeled extracellular domain of SRCL binds to the Lewisx- and Lewisa-containing ligands but not to the branched, unfucosylated oligosaccharides. Thus, SRCL shows selectivity for glycans bearing both terminal galactose and fucose residues.

A broader view of the binding selectivity of SRCL was obtained by probing a glycan array with fluorescently labeled extracellular domain. The array consists of over 150 different biotinylated oligosaccharides presented on streptavidin immobilized in polystyrene wells. The results reveal a striking selectivity for glycans containing terminal Lewisx trisaccharides, with no binding to sulfated or sialylated forms of these glycans (Fig. 6). Weaker binding was also observed for the isomeric Lewisa trisaccharide and to Lewisx, in which the galactose is substituted by N-acetylgalactosamine. In addition, very weak binding to blood group H substances was detected. The high degree of selectivity of SRCL is relatively unusual among C-type lectins.

Selectivity for Lewisx and Mechanism of Binding—Previous studies with the dendritic cell receptor DC-SIGN have revealed that this receptor, similar to SRCL, binds Lewisx and other glycans that bear terminal fucose and galactose residues, although it binds a broader range of such glycans. The primary binding site in the C-type CRD of DC-SIGN binds to the fucose moiety (24), whereas the primary binding site in SRCL has the...
hallmarks of a galactose-binding site. To clarify the nature of the primary monosaccharide-binding site, studies comparing the affinities of galactose, fucose, and Lewis\(^\text{a}\) trisaccharide were conducted (Fig. 7). The results, summarized in Table I, confirm that the affinity of SRCL for Lewis\(^\text{a}\) trisaccharide is much higher than the affinity for galactose. The affinity for fucose is roughly 4-fold lower than the affinity for galactose, suggesting that galactose is the primary site ligand. Comparison of \(\alpha\)-methyl galactoside and \(\alpha\)-methyl fucoside binding shows an even stronger preference for the galactose moiety, probably because the presence of the methyl group eliminates artifactual binding through the 1-hydroxyl group (27).

The availability of a crystal structure for a galactose-binding variant of the CRD from rat serum mannose-binding protein made it possible to model the binding site of SRCL. The conformation of the Lewis\(^\text{a}\) trisaccharide was derived from one of the several crystal structures that contain this oligosaccharide as part of a larger structure, and the galactose moiety was superimposed on the galactose residue in the CRD structure. Galactose occupies a primary binding site that involves coordination with Ca\(^{2+}\) and hydrophobic packing with a tryptophan residue (Fig. 8). The model shows that the fucose residue in the Lewis\(^\text{a}\) structure can be accommodated in the binding site with no steric clashes. The structure also suggests that the fucose might interact with the short segment-designated loop 5, consisting of the sequence Lys-Ala-Gly at residues 691–693. Mutants in which the sequence of the loop was modified to Arg-Pro-Gly, as found in the asialoglycoprotein receptor, or to Ala-Ala-Gly, to eliminate most potential side chain interactions, failed to change the affinity for Lewis\(^\text{a}\) compared with galactose (data not shown). These results suggest that interactions with loop 5 might be with backbone atoms rather than the side chains.

A weak binding interaction with the blood group H substances was also detected in the glycan array. Because these oligosaccharides do not contain terminal galactose residues, it was interesting to consider how they might be accommodated in the binding site. The fucose residue in these structures is attached to the 2 position of galactose, so the arrangement of coordination ligands with the 3- and 4-hydroxy groups need not be perturbed. As no crystal structure with the H-type glycans has been reported, it is not possible to develop a detailed model of where the fucose residue is located. However, modeling of a fucose residue in linkage to the 2-hydroxyl group of galactose suggests that it can be accommodated and that it might make energetically favorable contacts with loop 5 or other residues on the surface of the CRD. Thus, the binding and modeling results provide a consistent picture of galactose- and fucose-containing oligosaccharides interacting with SRCL through a primary galactose-binding site and secondary contacts with fucose.

**Endocytic Activity of SRCL**—Although SRCL binds selectively to Lewis\(^\text{a}\)-type structures, its binding activity overlaps with the binding activity of the asialoglycoprotein receptor. The presence of SRCL on endothelial cells suggests that it might interact with a subset of circulating glycoproteins from which terminal sialic acid has been removed. It was, therefore, of interest to examine the ability of SRCL to mediate uptake of such glycoproteins.

The cytoplasmic domain of SRCL contains the sequence Tyr-Lys-Arg-Phe, which is a potential target for binding of adapter proteins that direct receptors to clathrin-coated pits for endocytosis (29). Studies with other receptors suggest that, in ad-
Results presented here, as well as the evolutionary considerations discussed in the Introduction, suggest that other possible functions for SRCL need to be considered. However, DC-SIGN binds a much broader range of fucose-containing ligands (24). Indeed, a truncated form of SRCL lacking the CRD seems to retain the ability to bind micro-organisms, leading to the suggestion that the binding site lies on the collagen-like domain (2). In contrast, the collagen-like domain of serum mannose-binding protein and other collectins contain C-type CRDs, collagen-like domains and regions consisting of coiled coils of α helices (31). Because of the well-established roles of several of the collectins in innate immunity, a similar role has been suggested for SRCL (1, 2). Thus, SRCL differs from the collectins both in arrangement of domains and the functions of these domains.

The fact that bacteria and yeast can interact with SRCL is consistent with a role in innate immunity (1, 2). However, it is important to note that SRCL is found on endothelial cells rather than cells such as macrophages or dendritic cells that are usually associated with the innate immune response. In addition, the way that SRCL interacts with microbes is very different from the way that the collectins bind to them. The CRDs of the collectins fall into the subcategory of C-type lectins that bind to mannose, GlcNAc, and related sugars (3). They bind weakly to individual sugars and achieve selective binding to the surfaces of bacteria and fungi by interacting with repeating arrays of sugars in the walls of these micro-organisms (32). The data on binding selectivity presented here indicate that the CRD of SRCL falls into the other subcategory of C-type CRDs, which bind galactose and related sugars in the primary binding site. This specificity is not typical of pathogen-binding molecules. Indeed, a truncated form of SRCL lacking the CRD seems to retain the ability to bind micro-organisms, leading to the suggestion that the binding site lies on the collagen-like domain (2). In contrast, the collagen-like domain of serum mannose-binding protein interacts with serine proteases that activate complement (33). Thus, SRCL differs from the collectins both in arrangement of domains and the functions of these domains.

The preferred ligands for SRCL have adjacent terminal galactose and fucose residues. Thus, SRCL shares with DC-SIGN the ability to bind Lewisα-containing oligosaccharides (24). However, DC-SIGN binds a much broader range of fucose-
containing ligands, and the relative roles of fucose and galactose in the binding interaction are reversed. The different localizations of SRCL and DC-SIGN suggest that, despite their overlapping ability to bind the Lewis\(^x\) structure, they would probably target different glycoconjugate ligands.

The highly specific binding of SRCL to the Lewis\(^x\) trisaccharide suggests other possible roles for this receptor in the selective clearance of specific desialylated glycoproteins from the circulation or selective interaction of cells bearing Lewis\(^x\)-type structures with the vascular endothelium. These roles would be more analogous to the functions of the asialoglycoprotein receptor (34) and the selectin cell adhesion molecules (35). Similar to the hepatic asialoglycoprotein receptor, SRCL has the ability to bind asialo-orosomucoid. Differences in the number of receptors may explain why the liver receptor normally provides selective clearance of specific desialylated glycoproteins from circulation. SRCL might provide an alternative mechanism for receptors may explain why the liver receptor normally provides selective clearance of specific desialylated glycoproteins from circulation. SRCL might provide an alternative mechanism for clearance of some asialo-glycoprotein in asialoglycoprotein receptor-deficient mice (36, 37), although most asialoglycoproteins (which lack outer arm fucose residues) would not be taken up by this receptor.

The distribution of SRCL on vascular endothelial cells suggests parallels with the selectins, which bind to elaborated versions of the Lewis\(^x\) trisaccharide, bearing sialic acid and sulfate groups (35). The restricted binding to a specific glycan structure suggests possible roles in cell adhesion analogous to the leukocyte-endothelial interactions mediated by the selectins. E- and P-selectin are selectively expressed at sites of inflammation, whereas SRCL seems to be constitutively expressed on a wide variety of endothelial cells, although possible modulation of SRCL expression remains to be investigated. Knowledge of the glycan-binding selectivity of SRCL will also allow further study of possible glycoprotein and cellular target ligands.

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REFERENCES

1. Ohtani, K., Suzuki, Y., Eda, S., Kawai, T., Kase, T., Keshi, H., Sakai, Y., Fukuo, A., Sakamoto, T., Itabe, H., Suzutani, T., Ogasawara, M., Yoshida, I., and Wakamiya, N. (2001) J. Biol. Chem. 276, 44222–44228
2. Nakamura, K., Funakoshi, H., Miyamoto, K., Tokunaga, F., and Nakamura, T. (2003) Biochem. Biophys. Res. Commun. 296, 1028–1033
3. Drickamer, K. (1992) Nature 360, 183–186
4. Ishibashi, S., and Drickamer, K. (1994) J. Biol. Chem. 269, 15512–15519
5. Yoshida, T., Tsuruta, Y., Iwasaki, M., Yamane, S., Ochi, T., and Suzuki, R. (2003) J. Biochem. (Tokyo) 133, 271–277
6. Peiser, L., Mukhopadhyay, S., and Gorden, S. (2002) Curr. Opin. Immunol. 14, 123–128
7. Han, H.-J., Tokino, T., and Nakamura, Y. (1998) Hum. Mol. Genet. 7, 1039–1046
8. Grayeb, J., Kimura, H., Takahara, M., Hisung, H., Masui, Y., and Inouye, M. (1984) EMBO J. 3, 2437–2442
9. Foronjed, N., and Porath, J. (1975) FEBS Lett. 57, 187–191
10. Matsuda, A. (1977) J. Biol. Chem. 262, 10035–10038
11. Taylor, M. E., Bezouska, K., and Drickamer, K. (1992) J. Biol. Chem. 267, 1719–1726
12. Taylor, M. E., and Drickamer, K. (1993) J. Biol. Chem. 268, 399–404
13. Kaufman, R. J., Davies, M. V., Wasley, L. C., and Michnick, D. (1991) Nuc. Acids Res. 19, 4485–4490
14. Graham, F. L., and van der Eb, A. J. (1973) Virology 52, 456–467
15. Maddon, P. J., Littman, D. R., Godfrey, M., Maddon, D. E., Chess, L., and Axel, R. (1985) Cell 42, 93–104
16. Southern, P. J., and Berg, P. (1982) J. Mol. Appl. Genet. 1, 327–341
17. Stambach, N. S., and Taylor, M. E. (2003) Glycobiology 13, 401–410
18. Burnette, W. N. (1981) Anal. Biochem. 112, 205–208
19. Cohn, E. J., and Edsall, J. T. (1943) Proteins, Amino Acids and Peptides as Ions and Dipolar Ions, pp. 370–381, Reinhold, New York.
20. Bolton, A. E., and Hunter, W. M. (1973) Biochem. J. 133, 529–539
21. Feizi, T., Stoll, M. S., Yuen, C.-T., Chai, W., and Lawson, A. M. (1994) Methods Enzymol. 230, 484–491
22. Greenwood, F. C., Hunter, W. M., and Glover, J. S. (1963) Biochem. J. 89, 114–123
23. Kolattukudy, P. E., and Weis, W. I. (1995) J. Biol. Chem. 271, 6679–6685
24. Guo, Y., Feinberg, H., Conroy, E., Mitchell, D. A., Alvarez, R., Blixt, O., Taylor, M. E., Weis, W. I., and Drickamer, K. (2004) Nat. Struct. Mol. Biol. 11, 591–598
25. Mello, T. E., Halberg, D., and Drickamer, K. (1988) J. Biol. Chem. 263, 5468–5473
26. Treush, H. J., Costello, C. E., and Halsall, H. B. (1992) Biochem. J. 283, 105–112
27. Ng, K.-S., Drickamer, K., and Weis, W. I. (1996) J. Biol. Chem. 271, 663–672
28. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
29. Kraush, J. L. (2003) J. Cell Biol. 163, 283–288
30. Wragg, S., and Drickamer, K. (1999) J. Biol. Chem. 274, 35400–35406
31. Weis, W. I., and Drickamer, K. (1994) Structure (Lond.) 2, 1227–1240
32. Weis, W. I., Taylor, M. E., and Drickamer, K. (1998) ImmunoL Rev. 163, 19–34
33. Wallis, R., Shaw, J. M., Uitdehaag, J., Chen, C.-B., Torgersen, D., and Drickamer, K. (2004) J. Biol. Chem. 279, 14065–14073
34. Spikes, M. (1990) Biochemistry 29, 10009–10019
35. Vestweber, D., and Blanks, J. E. (1999) Physiol. Rev. 79, 181–213
36. Ishibashi, S., Hammer, R., and Herz, J. (1994) J. Biol. Chem. 269, 27603–27606
37. Tozawa, R., Ishibashi, S., Osuga, J., Yamamoto, K., Yagyu, H., Ohashi, K., Tamura, Y., Yahagi, N., Iizuka, Y., Okazaki, H., Harada, K., Gotoda, T., Shimano, H., Kimura, S., Nagai, R., and Yamada, N. (2003) J. Biol. Chem. 278, 12624–12628