L-selectin Interactions with Novel Mono- and Multisulfated Lewis* Sequences in Comparison with the Potent Ligand 3′-Sulfated Lewis*a*

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The cell adhesion molecule L-selectin binds to 3′-sialyl-Lewis (Le*) and -Le* and to 3′-sulfo-Le* and -Le* sequences. The binding to 3′-sialyl-Le* is strongly affected by the presence of 6-O-sulfate as found on oligosaccharides of the counter receptor, GlyCAM-1; 6-O-sulfate on the N-acetylgalactosamine (6-sulfation) enhances, whereas 6-O-sulfate on the galactose (6'-sulfation) virtually abolishes binding. To extend knowledge on the specificity of L-selectin, we have investigated interactions with novel sulfo-oligosaccharides based on the Lex pentasaccharide sequence. We observe that, also with 3′-sulfo-Le*, the 6-sulfation enhances and 6'-sulfation suppresses L-selectin binding. The 6′-sulfation without 3′-sialyl or 3′-sulfate gives no binding signal with L-selectin. Where the 6-sulfo,3′-sialyl-Le* is on an extended di-N-acetyllactosamine backbone, additional 6-O-sulfates on the inner galactose and inner N-acetylgalactosamine do not influence the binding. Although binding to the 6,3′-sulfo-Le* and 6′-sulfo,3′-sialyl-Le* sequences is comparable, the former is a more effective inhibitor of L-selectin binding. This difference is most apparent when L-selectin is in paucivalent form (predominantly di- and tetramer) rather than multivalent. Indeed, as inhibitors of the paucivalent L-selectin, the 3′-sulfo-Le* series are more potent than the corresponding 3′-sialyl-Le* series. Thus, for synthetic strategies to design therapeutic oligosaccharide analogs as antagonists of L-selectin binding, those based on the simpler 3′-sulfo-Le* (and also the 3′-sulfo-Le*) would seem most appropriate.

L-selectin, a carbohydrate-binding adhesion molecule on leukocytes that binds to saccharide ligands on high endothelial cells in post-capillary venules of lymph nodes, has a key role in the initial stages of leukocyte extravasation into peripheral lymph nodes and areas of acute and chronic inflammation (1, 2). Previous work with structurally defined oligosaccharides has shown that L-selectin binds to Lewis* (Le*) and Le* sequences sialylated or sulfated at position 3 of outer galactose with a preference for binding to 3′-sialyl-Le* over 3′-sialyl-Le*

(3) and a preference of 3′-sulfo-Le* and 3′-sulfo-Le* over the sialyl forms (4–6). The occurrence of 3′-sulfated forms of Le* and Le* has been documented on epithelial glycoproteins, and this led to the demonstration that sulfate can substitute effectively for sialic acid in ligands for the E- and L-selectins (4, 7, 8). Among these four sequences, the strongest binding signal is with the 3′-sulfo-Le* (6). These findings are important for the design of synthetic, potentially therapeutic analogs of the selectin ligands, as chemical synthesis of sulfated oligosaccharides is far more facile than of sialyl-oligosaccharides. O-glycosidic oligosaccharides with other sulfation patterns have been isolated from one of the counter-receptors of L-selectin, GlyCAM-1; these are heptasaccharides with 3′-sialyl-Le* capping groups containing 6-O-sulfate at the outer galactose (referred to as 6′-O-sulfation), at the penultimate N-acetylgalactosamine (6-O-sulfation), or at both of these positions (6′,6-O-sulfation) (9). We have demonstrated that the 6-sulfo,3′-sialyl-Le* sequence constitutes a strong ligand for L-selectin, particularly where the N-acetylatedneuraminic acid is de-N-acetylated, whereas the 6-sulfated analog is not bound (10, 24). Indeed, the addition of 6′-O-sulfate to the 6-sulfo,3′-sialyl-Le* sequence impairs the L-selectin binding.

Knowing that 3′-sulfation at galactose of Le* or Le* can substitute for 3′-sialylation in the formation of saccharide motifs recognized by the selectins, we have explored in the present study the reactivities of human L-selectin with a novel series of mono- and multisulfated Le* sequences in which the 3′-sialyl residue on Le* is replaced by 3′-O-sulfate, and we make a comparison with reactivity toward the 3′-sulfo-Le* sequence, which is among the most potent L-selectin ligands thus far described, and also with reactivity toward the 6′-sulfo-Le*. We report here results that reveal an advantage of 3′-sulfation over 3′-sialylation of Le* with respect to inhibitory activity toward L-selectin binding and the deleterious effect of 6′-sulfation of Le* with respect both to binding and inhibitory activity toward L-selectin binding. We also examine L-selectin binding to a novel trisulfated sequence, 3′-sialyl,6′-sulfo-di-N-acetyl lactosamine, with two additional sulfates, one at the inner galactose and another at the inner N-acetylgalactosamine residue (both at position 6). We show that the additional sulfates along the extended backbone do not influence the binding signal.

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The abbreviations used are: Le*, Le*, Lewis*, and Lewis.

MATERIALS AND METHODS

Oligosaccharides, Neoglycolipids, and Glycosylceramides—The carbohydrate sequences investigated are shown in Table I. The following were synthesized chemically: the 3′-sulfo-Le* (11); the 3′-sulfo-Le*, the 6′,3′-sulfo-Le*; the 6,3′-sulfo-Le*; the 6′-sulfo-Le* (12); and also the
3′-sialyl-Lea pentasaccharide,2 GSC151, (13). These were purified by high pressure liquid chromatography using a TSK-Gel® amide 80 column (Tosohas) (6). Lacto-N-tetraose and lacto-N-neo-tetraose were purchased from Dextra. The oligosaccharide designated C4U was prepared by keratanase II digestion of bovine articular keratan sulfate essentially as described by Brown et al. (14). The oligosaccharide designated Fuc-C4U was derived from C4U by fucosylation using milk oligosaccharides, the term pentasaccharide is used to denote the fucosyl tetrasaccharide backbones that they share.

For ease of comparison of the sialyl-Lea and -Lea with the sulfo-Lea and -Lea oligosaccharides, the term pentasaccharide is used to denote the fucosyl tetrasaccharide backbones that they share.

G. M. Brown, manuscript in preparation.

TABLE I
Carbohydrate sequences investigated

| Designation   | Abbreviation | Sequence                   |
|---------------|--------------|----------------------------|
| 3′-sulfo-Lea  | 3′Su-Lea     | Galβ1-3GlcNAcβ1-3Galβ1-3Glc [3,1,4] HSO3 Fucα |
| 3′-sulfo-Lea  | 3′Su-Lea     | Galβ1-4GlcNAcβ1-3Galβ1-4Glc [3,1,3] HSO3 Fucα |
| 6′-sulfo-Lea  | 6′Su-Lea     | Galβ1-4GlcNAcβ1-3Galβ1-4Glc [1,1,3] Fucα |
| 6′,3′-sulfo-Lea | 6′,3′Su-Lea | Galβ1-4GlcNAcβ1-3Galβ1-4Glc [3,1,3] HSO3 Fucα |
| 6,3′-sulfo-Lea | 6,3′Su-Lea  | Galβ1-4GlcNAcβ1-3Galβ1-4Glc [3,1,3] HSO3 Fucα |
| 3′-sialyl-Lea | 3′S-Lea      | Galβ1-4GlcNAcβ1-3Galβ1-4Glc [2,3,1,3] NeuAcα Fucα |
| 6′-sulfo,3′-sialyl-Lea | 6′Su,3′S-Lea | Galβ1-4GlcNAcβ1-3Galβ1-4Glc [2,3,1,3] NeuAcα Fucα |
| 6-sulfo,3′-sialyl-Lea | 6Su,3′S-Lea | Galβ1-4GlcNAcβ1-3Galβ1-4Glc [2,3,1,3] NeuAcα Fucα |
| 6,6-sulfo,3′-sialyl-Lea | 6,6Su,3′S-Lea | Galβ1-4GlcNAcβ1-3Galβ1-4Glc [2,3,1,3] NeuAcα Fucα |
| C4U           | C4U          | Galβ1-4GlcNAcβ1-3Galβ1-4GlcNac [2,3] NeuAcα |
| Fuc-C4U       | Fuc-C4U      | Galβ1-4GlcNAcβ1-3Galβ1-4GlcNac [2,3] NeuAcα |
| Lacto-N-tetraose | LNT         | Galβ1-3GlcNAcβ1-3Galβ1-3Glc |
| Lacto-N-neo-tetraose | LNTN        | Galβ1-4GlcNAcβ1-3Galβ1-4Glc |
L-selectin Interactions with Multisulfated Oligosaccharides

3'-sulfated-Leα (3'Su-Leα) and 3'-sialyl-Leα.

L-selectin Binding and Inhibition Assays—For direct binding experiments (10), purified lipid-linked oligosaccharides (glycolipids and neo-glycolipids) were immobilized on microwells (Falcon 3912). About 30% of the lipid-binding oligosaccharides added were retained in the microwells under the binding assay conditions (6). Fifty ng of multivalent L-selectin IgG chimeras was applied per well using as diluent 10 mM Tris buffer, pH 7.4, containing 150 mM NaCl and 2 mM CaCl2. As in previous experiments with E-selectin (7), binding intensity of L-selectin was of the same order to the 3'-sialy-Leα sequence in the form of a glycosylceramide or a neoglycolipid (not shown). For inhibition experiments, the immobilized oligosaccharides used were either the lipid-linked 6-sulfo,3'-sialyl-Leα or 6,3'-sulfo-Leα (100 pmol added per well) and 10 ng of the multivalent L-selectin or 50 ng of the paucivalent L-selectin were added per well. These levels of L-selectin were used in the inhibition experiments as they gave comparable binding signals with the two immobilized ligands. Serial dilutions of liposomes containing lipid-linked oligosaccharides were used as inhibitors. These consisted of cholesterol:lecithin:lipid-linked oligosaccharides at ratios of 0.4:0.4:1 by weight.

RESULTS

Binding of Multivalent L-selectin to Mono- and Multisulfated Sequences—In accord with earlier observations (6), the 3'-sulfated-Leα sequence was bound by human L-selectin but less strongly than 3'-sulfo-Leα. However, the 6'-sulfo-Leα was not bound (Fig. 1A). The 6-O-sulfation of 3'-sulfo-Leα gave 6,3'-sulfo-Leα elicited enhanced L-selectin binding, whereas the 6'-sulfated analog 6,3'-sulfo-Leα consistently showed some diminution of binding. The binding signal observed with the 6,3'-sulfated-Leα sequence was of the same order as that observed with the 6-sulfo,3'-sialyl-Leα (Fig. 1C). Thus, 6-sulfation, but not 6'-sulfation, has a potentiating effect on human L-selectin binding not only to the 3'-sialyl-Leα, as shown previously (Ref. 10 and Fig. 1B), but also to the 3'-sulfo-Leα sequence. The hindering effect of the 6'-O-sulfation was less pronounced on 3'-sulfo-Leα than on the 3'-sialyl-Leα analog investigated previously (Fig. 1B) (10).

Binding experiments with the oligosaccharide C4U and its 3'-fucosylated analog, Fuc-C4U, clearly showed that the presence of the fucose residue is essential for L-selectin binding to the 3'-sialyl,6-sulfated di-N-acetyllactosamine backbone (Fig. 1D). The binding signals with the Fuc-C4U and the 6-sulfo,3'-sialyl-Leα were similar, indicating that the additional sulfates on the internal galactose and N-acetylgalactosamine residues do not influence the L-selectin binding signal.

Inhibition of the Binding of Multivalent L-selectin—Inhibition experiments were performed with the multivalent L-selectin using as immobilized ligands the 6-sulfo,3'-sialyl-Leα or the 6,3'-sulfo-Leα (Figs. 2 and 3, A and B); all of the acidic compounds of the Leα series tested gave some inhibition of binding. The nonsulfated lacto-N-tetraose gave no inhibition. The 6,3'-sulfo-Leα (IC50 1.4 × 10−7 and 1.5 × 10−7 M with the two immobilized ligands) was approximately 3 and 5 times more active as an inhibitor than the 6-sulfo,3'-sialyl-Leα (5.1 × 10−7 and 6.9 × 10−7 M). The least active inhibitors were the nonsulfated 3'-sialyl-Leβ (IC50 3.7 × 10−6 and 6.8 × 10−6 M), the 6-sulfo-Leβ (3.1 × 10−6 and 2.0 × 10−6 M) and the 6-sulfo,3'-sialyl-Leα (7.4 × 10−6 and 1.0 × 10−6 M). Fine comparisons of the inhibitory activities of the relatively potent sequences were difficult as inhibition curves were often closely spaced or partially overlapping as in Fig. 2B.

Inhibition of the Binding of Paucivalent L-selectin—Inhibition of binding experiments using the paucivalent L-selectin was performed (Figs. 3, C and D, and 4) to discriminate more clearly (6) between relatively high and low affinity inhibitors and under conditions that may possibly simulate situations in vivo where L-selectin is relatively sparsely expressed. The degree of inhibition of selctin binding depended on the immobilized ligand used; binding to the 6,3'-sulfo-Leα was harder to inhibit than to the 6-sulfo,3'-sialyl-Leα (Fig. 3, C and D). Here the 3'-sialyl-Leα series were poorer inhibitors overall than the corresponding 3'-sulfo-Leα analogs and gave no significant inhibition when the 6,3'-sulfo-Leα was used as the immobilized ligand (Fig. 3D); when the 6-sulfo,3'-sialyl-Leα was used as the immobilized ligand (Fig. 3C), only the homologous oligosaccha-
ride sequence showed reasonable inhibition (IC$_{50}$ 1.6 × $10^{-6}$ M). In contrast, among the oligosaccharides in which there was 3'-sulfate instead of 3'-sialyl, all inhibited the selectin binding to the two immobilized ligands. The 6'-sulfo-Le$^x$ was not inhibitory. The 3'-sulfo-Le$^x$ and the 6,3'-sulfo-Le$^x$ stood out as superior inhibitors with IC$_{50}$ values of 1.4 × $10^{-7}$ and 5.0 × $10^{-7}$ M, and 5 × $10^{-8}$ and 2.7 × $10^{-7}$ M, respectively. Also included in the present inhibition experiments for comparison was the 3'-sulfo-Le$^x$ sequence; its potency was intermediate between that of 3'-sulfo-Le$^x$ and 6,3'-sulfo-Le$^x$ (Fig. 3, C and D).

**DISCUSSION**

It is clear from the binding experiments described here, first, that, in contrast to 3'-sulfation of Le$^x$, which supports L-selectin reactivity, the 6'-sulfation does not elicit a detectable binding signal. Second, the L-selectin reactivities of 3'-sulfo- and 3'-sialyl-Le$^x$ are similarly influenced by the addition of 6'-sulfate or 6-sulfate. Whereas 6'-sulfation has a negative effect, 6-sulfation has an enhancing effect on L-selectin binding to the clustered, immobilized 3'-sulfo- and 3'-sialyl-Le$^x$ series. Thus the 6-sulfate seems to be a part of the recognition motif for L-selectin, whereas 6'-sulfate is not. Moreover, its presence partially hinders the recognition of the 3'-sialyl- or 3'-sulfo-Le$^x$ by the selectin. The enhancement of L-selectin binding in the presence of 6-sulfation was substantial for both the 3'-sulfo- and the 3'-sialyl-Le$^x$ sequences, but the negative effect of 6'-sulfation was less pronounced with the 3'-sulfo-Le$^x$ than with the 3'-sialyl-Le$^x$ sequence investigated previously (10). Third, whereas the 6-sulfate on the subterminal N-acetyllactosamine of the Le$^x$ sequence is clearly a part of the recognition sequence for L-selectin, additional sulfates along the di-N-acetyllactosamine backbone are not recognized. Fourth, the presence of the 3'-linked fucose on 6-sulfo,3'-sialyl-Le$^x$ is essential for L-selectin binding. In accord with this finding is an earlier report that L-selectin binding is abolished by modification of the fucose in the 3'-sialyl-Le$^x$ sequence by removal of oxygen at position 2, 3, or 5 (18). The presence of the fucose is apparently less critical in the 3'-sulfo-Le$^x$ sequence as it has been observed that the defucosylated 3'-sulfo-Le$^x$ tri- and tetrasaccharides are bound by L-selectin, albeit less strongly than the 3'-fucosyl analogs (4, 5).

The inhibition experiments described here reveal subtle differences in L-selectin reactivities that are not apparent in the binding experiments, namely a slightly greater binding affinity toward the 3'-sulfo than the 3'-sialyl-Le$^x$. The 6-sulfated 3'-sulfo-Le$^x$ sequence had a greater inhibitory activity than the 6-sulfated 3'-sialyl-Le$^x$, although the binding signals they elicited were similar. The differences were more apparent in inhibition experiments where the paucivalent L-selectin was used;
Inhibitor concentration (M) 

Fig. 4. Inhibition of the binding of the paucivalent human L-selectin by lipid-linked oligosaccharides of the 3'-sulfo-Le^a and 3'-sialyl-Le^a series, displayed on liposomes. The inhibition of the binding of 50 ng/well of the paucivalent L-selectin was assayed as described under “Materials and Methods.” For clarity, results with selected compounds are shown; the IC_{50} values for all of the tested compounds are shown in Fig. 3. The immobilized ligand in A and C was the 6-sulfo-3'-sialyl-Le^a, and in B and D it was the 6,3'-sulfo-Le^a. Specific binding signals were 0.8–1.2 (A_{530 nm}) in the absence of inhibitor after subtracting the signals in control wells containing carrier lipid only. Results are expressed as means in duplicate wells with the range indicated by error bars. See Table I for abbreviations.

these experiments clearly showed differences in the ease of inhibition of L-selectin binding depending on the immobilized ligand used, and they showed that inhibitory activities of the 3'-sulfo-Le^a series were greater than those of the 3'-sialyl-Le^a series. Collectively, these data, together with the earlier finding (4) that the 3'-sulfo- but not the 3'-sialyl-lactosamine backbone (in the absence of fucose) elicits a binding signal with L-selectin, indicate that the 3'-sulfation at the galactose of Le^a creates a higher affinity ligand than the 3'-sialylation and that L-selectin has overall a higher affinity toward the 3'-sulfo- and 6,3'-sulfo-Le^a sequences than the corresponding 3'-sialyl-Le^a analogs.

The inhibition experiments were performed here using as inhibitors oligosaccharides linked to a lipid and displayed on liposomes as it was found earlier that about 10,000-fold less oligosaccharide material is required compared with the free oligosaccharides. This is in accord with results from other groups who have examined free oligosaccharides as inhibitors of L-selectin binding; the reported IC_{50} values range from 0.2 to 6 mM (19-22). When examined in the oligomeric state on dendrimers, IC_{50} values in the 1–10 μM range were reported (23). A general feature that emerges from our inhibition experiments using L-selectin in the multivalent state and those of other groups who have used the multivalent L-selectin is that differences in inhibitory activities of the various acide Le^a and Le^a oligosaccharides are less marked. Also, in the multivalent assay, the differing ease of inhibition of L-selectin binding to different immobilized ligands is less readily discernible.

The modification to the inhibition assay that we have introduced serves to establish a hierarchy of inhibitory activities that may be relevant in in vivo situations where the display of L-selectin is relatively sparse. Thus, the inhibition assay with paucivalent L-selectin establishes that the 3'-sulfo-Le^a series and also the 3'-sulfo-Le^a are more effective inhibitors of the L-selectin binding than the 3'-sialyl-Le^a analogs. Among these, the 6,3'-sulfo-Le^a and the monosulfated 3'-sulfo-Le^a are the most potent inhibitors of L-selectin binding among the oligosaccharide sequences so far tested. The former is only marginally better. Therefore, from the point of view of synthetic strategies for the design of therapeutic oligosaccharide analogs, those based on the relatively simple structure 3-sulfo-Le^a and -Le^a would seem the most appropriate.

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REFERENCES
1. Rosen, S. D. (1993) Histochemistry 100, 185–191
2. Tedder, T. F., Steeber, D. A., and Pizcueta, P. (1995) J. Exp. Med. 181, 2259–2264
3. Berg, E. L., Magnani, J., Warnock, R. A., Robinson, M. K., and Butcher, E. C. (1992) Biochem. Biophys. Res. Commun. 184, 1048–1055
4. Green, P. J., Tomatani, T., Watanabe, T., Miyazaki, M., Hasegawa, A., Kiss, M., Stoll, M. S., and Feizi, T. (1992) Biochem. Biophys. Res. Commun. 188, 244–251
5. Green, P. J., Yuen, C.-T., Childs, R. A., Chai, W., Miyazaki, M., Lemoine, R., Lubineau, A., Smith, B., Ueno, H., Nicolau, K. C., and Feizi, T. (1995) Glycobiology 5, 29–38
6. Galustian, C., Childs, R. A., Yuen, C.-T., Hasegawa, A., Kiss, M., Lubineau, A., Shaw, G., and Feizi, T. (1997) Biochemistry 36, 5260–5266
7. Yuen, C.-T., Lawson, A. M., Chai, W., Larkin, M., Stoll, M. S., Stuart, A. C., Sullivan, F. X., Ahern, T. J., and Feizi, T. (1992) Biochemistry 31, 9126–9131
8. Chai, W., Feizi, T., Yuen, C.-T., and Lawson, A. M. (1997) Glycobiology 7, 861–872
9. Hemmerich, S., and Rosen, S. D. (1994) Biochemistry 33, 4830–4835
10. Galustian, C., Lawson, A. M., Komba, S., Ishida, H., Kiss, M., and Feizi, T. (1997) Biochem. Biophys. Res. Commun. 240, 748–751
11. Lubineau, A., Le-Gallucic, J., and Lemoine, R. (1994) Bioorg. Med. Chem. Lett. 2, 1143–1151
12. Auge, C., Dagron, F., Lemoine, R., Le Narcor, C., and Lubineau, A. (1997) in Carbohydrate Mimics: Concepts and Methods (Chapleur, V., ed) pp. 365–383, Verlag Chemie, Weinheim, Germany
13. Hasegawa, A., Ando, T., Kameyama, A., and Kiss, M. (1992) J. Carbohydr. Chem. 11, 645–658
14. Brown, G. M., Huckerby, T. N., Morris, H. G., Abram, R. L., and Nieduszynski, I. A. (1994) Biochemistry 33, 4836–4846
15. Tang, P. W., Gooi, H. C., Hardy, M., Lee, Y. C., and Feizi, T. (1985) Biochem. Biophys. Res. Commun. 132, 474–480
16. Feizi, T., Stoll, M. S., Yuen, C.-T., Chai, W., and Lawson, A. M. (1994) Methods Enzymol. 230, 484–519
17. Komba, S., Ishida, H., Kiss, M., and Hasegawa, A. (1996) Bioorg. Med. Chem. 4, 1833–1847
18. Brandley, B. K., Kiso, M., Abbas, S., Nikrad, P., Srivasatava, O., Foxall, C., Oda, Y., and Hasegawa, A. (1993) Glycobiology 3, 633–641
19. Manning, D. D., Bertozzi, C. R., Pohl, N. L., Rosen, S. D., and Kiesling, L. L. (1995) J. Org. Chem. 60, 6254–6255
20. Bertozzi, C. R., Fukuda, S., and Rosen, S. D. (1995) Biochemistry 34, 14271–14276
21. Sanders, W. J., Katsumoto, T. R., Bertozzi, C. R., Rosen, S. D., and Kiesling, L. L. (1996) Biochemistry 35, 14862–14867
22. Koenig, A., Jain, R., Vig, R., Norgard, S. K., Matta, K. L., and Varki, A. (1997) Glycobiology 7, 79–93
23. Roy, R., Park, W. K. C., Zanini, D., Foxall, C., and Srivastava, O. P. (1997) Carbohydr. Lett. 2, 259–266
24. Komba, S., Galustian, G., Ishida, H., Feizi, T., Kannagi, R., and Kiso, M. (1999) Angew. Chem. Int. Ed. Engl. 38, 1131–1133
25. Berg, E. L., Magnani, J., Warnock, R. A., Robinson, M. K., and Butcher, E. C. (1992) Biochem. Biophys. Res. Commun. 184, 1048–1055
26. Tedder, T. F., Steeber, D. A., and Pizcueta, P. (1995) J. Exp. Med. 181, 2259–2264
27. Komba, S., Ishida, H., Kiso, M., and Hasegawa, A. (1993) Biochem. Biophys. Res. Commun. 240, 658–664
28. Komba, S., Galustian, G., Ishida, H., and Kiso, M. (1992) Angew. Chem. Int. Ed. Engl. 31, 79–93