Polymerized Liposome Assemblies: Bifunctional Macromolecular Selectin Inhibitors Mimicking Physiological Selectin Ligands†

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Received December 22, 2000

ABSTRACT: Monomeric sialyl Lewis^X (sLe^x) and sLe^x-like oligosaccharides are minimal structures capable of supporting selectin binding in vitro. However, their weak binding interactions do not correlate with the high-affinity binding interactions witnessed in vivo. The polyvalent display of carbohydrate groups found on cell surface glycoprotein structures may contribute to the enhanced binding strength of selectin-mediated adhesion. Detailed biochemical analyses of physiological selectin ligands have revealed a complicated composition of molecules that bind to the selectins in vivo and suggest that there are other requirements for tight binding beyond simple carbohydrate multimerization. In an effort to mimic the high-affinity binding, polyvalent scaffolds that contain multicomponent displays of selectin-binding ligands have been synthesized. Here, we demonstrate that the presentation of additional anionic functional groups in the form of sulfate esters, on a polymerized liposome surface containing a multimeric array of sLe^x-like oligosaccharides, generates a highly potent, bifunctional macromolecular assembly. This assembly inhibits L-, E-, and P-selectin binding to GlyCAM-1, a physiological ligand better than sLe^x-like liposomes without additional anionic charge. These multivalent arrays are 4 orders of magnitude better than the monovalent carbohydrate. Liposomes displaying 3′-sulfo Lewis^X-like oligosaccharides, on the other hand, show slight *loss* of binding with introduction of additional anionic functional groups for E- and P-selectin and negligible change for L-selectin. The ability to rapidly and systematically vary the composition of these assemblies is a distinguishing feature of this methodology and may be applied to the study of other systems where composite binding determinants are important for high-affinity binding.

Localized recruitment of leukocytes into tissues at sites of injury, infection, or disease is central to an inflammatory response. This is achieved through sequential adhesion events involving leukocyte and endothelial cell adhesion molecules belonging to the selectin, integrin, and immunoglobulin gene families (reviewed in refs *1*−*15*). Leukocyte tethering to and rolling on vascular endothelium is the first in this series of adhesion events and is critical for a successful inflammatory response. Three calcium-dependent carbohydrate-binding proteins, L-, E-, and P-selectin, mediate this initial step and have attracted significant attention as potential targets for anti-inflammatory therapeutics (*4*, *14*, *16*). L-Selectin is constitutively expressed on most circulating leukocytes and binds to sulfated carbohydrate ligands on activated endothelium and to carbohydrate ligands on leukocytes. E-Selectin is synthesized by activated endothelium in response to inflammatory mediators. P-Selectin exists preformed in storage granules and is translocated to the surface of activated platelets and endothelium during inflammation. E- and P-selectin both bind to carbohydrate ligands on leukocytes.

Five well-characterized selectin ligands, GlyCAM-1 (*17*), CD34 (*18*, *19*), Podocalyxin (*20*), PSGL-1 (*21*), and MadCAM-1 (*22*), are cell surface or secreted glycoproteins containing sialomucin domains that are heavily O-glycosylated and present multivalent arrays of sLe^x,[1] sulfated sLe^x, or sulfo-Le^x capped oligosaccharides (*23*−*26*). Although all three selectins bind to sLe^x in vitro (*27*), and early selectin inhibitors were modeled after this structure (*28*), detailed biochemical analyses of selectin ligands have illustrated a much more complicated composition of the

† This work was supported in part by DOE CRADA Grant BG92-173(00) and NIH Grant R4 AI 43789A, through U.S. Department of Energy Contract DE-AC03-76SF00098.

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[1] Abbreviations: sLe^x, sialyl Lewis X; 3′-sulfo-Le^x, 3′-sulfated Lewis X; GlyCAM-1, glycosylation-dependent cell adhesion molecule 1; PSGL-1, P-selectin glycoprotein ligand 1; MAdCAM-1, mucosyl addressin cell adhesion molecule 1; PCDA, 10,12-pentacosadiynoic acid; EtOAc, ethyl acetate; IPA, isopropyl alcohol; THF, tetrahydrofuran; MeOH, methyl alcohol; TEA, triethylamine; PDA, polydiacetylene; FAB, fast atom bombardment; Le^a, Lewis^a; Le^x, Lewis^x; NHS, *N*-hydroxysuccinimide.

10.1021/bi002921s CCC: $20.00
Published on Web 04/26/2001