Abstract

The interaction of heparin (HP) with the cell-surface components of a human uterine epithelial carcinoma cell line (RL95) was studied. Binding of [3H]HP to cell surfaces was saturable in a dose- and time-dependent manner. HP and certain forms of heparan sulfate (HS) efficiently compete for [3H]HP binding. In contrast, other glycosaminoglycans, such as chondroitin sulfate, keratan sulfate, hyaluronic acid, and dermatan sulfate, do not compete for binding to these sites. Scatchard analysis revealed that [3H]HP bound to these sites with an apparent KD of 0.7-0.9 microM and a binding capacity of 9 x 10(6) sites/cell to attached cells. EDTA-detached cells displayed a similar apparent KD, but an approximately 2-fold increase in binding capacity. Protease digestion of cells on ice markedly reduced [3H]HP binding, indicating that these binding sites were associated with proteins. In contrast, heparinase treatment of cells stimulated binding by approximately 2-fold, indicating that a large fraction of these binding sites were occupied with endogenous ligand. We examined the structural features of HP/HS required for HP/HS binding. O-Sulfation, substitution of amino groups, and, to a lesser extent, the presence of carboxyl groups were important recognition features of HP/HS by cell-surface HP/HS-binding sites. N-Sulfation was not required. Photoaffinity labeling with 125I-sulfosuccinimidyl 2-(p-azidosalicylamido)-ethyl-1, 3-dithiopropionate-HP was used to identify HP/HS-binding proteins on RL95 cell surfaces. Proteins with M(r) values of 14,000-18,500 and 31,000 were photolabeled at the surfaces of attached cells. Photolabeling was blocked by the addition of excess HP, but not chondroitin sulfate. Additional proteins with M(r) values greater than 31,000 were photolabeled specifically on EDTA-detached cells. Moreover, the M(r) 14,000-18,500 and 31,000 proteins were retained on the EDTA-detached cells. These observations indicated that certain cell-surface HP/HS-binding proteins were not exposed when cells were attached to substrata. Proteins of similar M(r) values as the photolabeled components as well as many additional proteins were identified by heparin-agarose chromatographic selection of extracts of cells labeled metabolically with [35S]methionine or vectorially with Na125I at the cell surface. Fragments of cell-surface HP/HS-binding proteins were released from intact RL95 and mouse uterine epithelial cells by mild trypsinization and isolated by heparin-agarose affinity chromatography. Three peptides with M(r) values between 6000 and 14,000 required greater than 0.5 M salt for elution from heparin-agarose, retained HP binding activity in a 125I-HP gel overlay assay, and selectively bound [3H]HP in a solid-phase binding assay.