Glycosaminoglycan model glass substrates and cancer cell interactions

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ABSTRACT

We have analyzed the the static adhesion of three cancer cell lines of varying metastatic potential to surfaces functionalized with glycosaminoglycans (GAGs) in relation to GAG charge per dimer and GAG chain length. Four different GAGs (heparan sulfate (HS), chondroitin sulfate A (CSA), chondroitin sulfate C (CSC) and keratan sulfate (KS)) were deposited on glass surfaces that had been previously coated with 3-aminopropyltriethoxy-silane (APTES), and the substrates were used in static adhesion experiments. GAGs were strongly bonded to the silanized glass cover slips. The best substrate for adhesion was HS while KS was the least preferred substrate. The observed adhesion of whole cancer cells showed that a functional dependence may exist with the number of charges per dimer of the polyelectrolyte to which they are attaching and that this dependence takes the form of a linear function that increases with the number of charges per dimer.

Keywords: glycosaminoglycan, adhesion, polysaccharide, glycobiology, biopolymer

1 INTRODUCTION

The study of the processes relating glycobiology and cancer will have increased interest in coming years. The analysis included here shows how experiments perfomed with substrates containing controlled carbohydrate composition, organization and orientation are useful for investigations in glycobiology. Characterization of the adhesion processes in which cancer cells make use of the polysaccharide chains of proteoglycans -GAGs- is of interest given the cellular localization of these molecules. We have focused here on the interaction of the cells with heparan, keratan and chondroitin sulfates. After deposition, the molecular structure of the functionalized glass surface can be understood as a reduced section of the outer areas of the extracellular glycocalyx or the extracellular matrix (ECM) containing proteoglycans.

Normally, methods to deposit biomolecules have consisted in growing endothelial cells on surfaces and then eliminating part of the biological material via lysis [1]. This artificial model has permitted testing for adhesion between cancerous cells and GAGs found in the ECM. However, that method cannot study individualized interactions between cells and molecular species of choice. We are using a new technique [2] that facilitates this analysis. The hypothesis that the adhesion of whole cancer cells to GAG substrates is a function of polysaccharide charge per dimer and chain length was proposed and tested.

2 EXPERIMENTAL METHODS

The experimental methods for glass surface modification [2,3] and cell adhesion experiments [4] have been described elsewhere. Briefly, glass surfaces were modified in a two-step procedure. First, silanization of the glass surface with APTES was performed producing a cover slip with an NH_2 terminated submonolayer or monolayer. After silanization, GAGs were deposited by submersion. The APTES substrates were incubated for 24 hours at room temperature in solutions of 0.1 μg/ml of HS, KS, CSA and CSC in PBS with $NaBH_3CN$ at a concentration of 3 μg/ml.

BT20, a moderately metastatic breast cancer cell line, MCF7 a non-metastatic breast cancer cell line, and A431, a highly metastatic epidermoid skin carcinoma cell line, were selected because of their different metastatic activity.

Cell adhesion assays were performed using confluently grown tumor cells, in two separate groups: non-treated cells and cells that were resuspended in a solution of complete media containing 10 μg/ml of heparin in heparin containing media. Cells were detached from culture flasks with trypsin and resuspended in the corresponding complete media for non-treated cells or in heparin containing media for specific assays with heparin. Cells resuspended in heparin containing solution were incubated for 30 minutes in an incubator prior to the assay. Cells then were transferred GAGs coated cover slips placed on the bottom of Petri dishes. Deposited cells were allowed to incubate at 37°C for 2 hours for A431 and BT20 cells and 8 hours for MCF7 cells. Seeding time was determined by comparing the number of cells adhered at 30 minutes, 1, 2 and 4 for hours (for A431 and BT20) and between 4, 6, 8 and 10 hours for MCF7.

The number of cells originally deposited on each cover slip was 2.5×10^5. Cells were carefully washed with PBS kept at room temperature to remove non-adherent cells. The remaining cells were detached using 250 μl of a solution of PBS-EDTA and then counted. For each experimental group, the results are expressed as the mean percentage (+/-

SD) of bound tumor cells in 9 cover slips. GAG densites on the surfaces where calculated by radiolabeling methods and the substrates were analyzed by ellipsometry (data not shown) indicating that a monolayer of product was present. Values are given after adjusting for equal surface density of GAGs.

3 RESULTS AND DISCUSSION

Previously we have shown[4] that the adhesion of cells differs for different GAGs substrates. In particular, non-treated and heparin treated cells showed little affinity for attachment to KS substrates compared to other GAGs, showing an anti-adhesive property that has been observed and established previously [5]. It was also shown that heparin had the effect of reducing the adhesion of the cells to all substrates in all cell lines with respect to the levels of non-treated cells, with few exceptions. Also, all cells showed preferential adhesion to heparan sulfate substrates and HS can be considered a good substrate for the adhesion of non-treated cells. These results indicated that it is likely that the adhesion levels may be related to the total electronic charge present in the molecules.

The basic idea is to determine if adhesion levels of the cells have some type of underlying functional dependence on some of the physical and chemical properties characterizing glycosaminoglycans. Essentially, the analysis that follows will focus on the electronic charge per dimer of glycosaminoglycan and its chain length, measured using the number of dimers that constitute the polysaccharide backbone. Here, then, we want to address the question of how the adhesion of cells differs for different GAGs substrates depending on the dimer charge, sulfation levels and possibly chain length of the molecules

It has been known for some time that charge interaction between glycosaminoglycans and proteins may require conformational changes in proteins, and that these interactions increase with the length and charge density of the molecules [6]. It is also known that if L-iduronic acid is present (for instance in HS chains), the binding has higher affinity than when it is not present. The results presented here indicate that the adhesion of cancer cells may functionally depend on the number of charges per dimer of the polyelectrolyte to which they are attaching and that this dependence takes the form of a linear function that increases with the number of charges of the dimer.

Specific parameters of this linear function appear to be cell line dependent and are probably modulated by factors related to cell surface density of ligands. In addition, as a secondary hypothesis, a more subtle relationship between cell adhesion and length of the polysaccharide chain may exist, but this relationship is not as clear as the previous one. A third aspect to be noted is that the presence or absence of glucuronic acid and the sulfation of the glucosamine residue affect adhesion levels but that consideration is implicitly included in the previous considerations.

The hypothesis then can be expressed in the following form: for a given density of cell surface receptors that bind to glycosaminoglycans and a given density of GAG ligands, cell adhesion to the polysaccharides is linearly dependent on the number of charges per dissacharide in the chain and also probably dependent on the length of the chain.

For the analysis that follows, the number of disaccharides (dimers) and charges per dimer of GAGs are as follows: HS (dimers = 22, charges = 2.5, sulfate groups = 1.5) ; KS (dimers = 32, charges = 0.5, sulfate groups = 0.5); CSA (dimers = 54, charges = 2.0, sulfate groups = 1); CSC (dimers = 131, charges = 1.75, sulfate groups = 0.75). For this calculation classical estimates of levels of sulfation given by Lindahl [7] were used.

The total number of charges directly depend on sulfation levels, given that except for the charge contribution of the COO^- groups of the glucuronic or iduronic acids in heparan and chondroitins, the rest of the contribution to the charge is due to the presence of the SO_3^- groups. The existence of other charged groups present on the polysaccharides is uncommon. The calculations have been made for the commercial products used during the experiments and represent a fair, good average estimate for the polysaccharide length and charge. Small deviations (+/- 0.25 charges/dimer) of these values do not change the general trend that supports the hypothesis of linear dependence of adhesion with charge per dimer.

In order to show the proposed dependence of the adhesion with charge per dimer, an individualized plot of adhesion *versus* sulfate and chain length is presented in Figure 1 for each cell line and condition. It must be clarified that no attempt has been made to obtain the precise mathematical form of the dependence, for any case. Rather, a simple linear best fit has been plotted to support the central hypothesis in the case of the adhesion *versus* charge diagrams. The inclusion in these results of adhesion data collected using more polysaccharide substrates could give enough information for the fitting values and extraction of the precise form of the mathematical relation. However, as shown, it has been possible to give, qualitatively, the approximate form of the relation and then provide a rationale for it. Figure 1 (left panel) shows a plot of the adhesion *vs.* sulfate per dimer for MCF7 (bottom), BT20 (middle) and A431 (top) of non treated cells. The fact that CSC and CSA have similar charge values per dimer but differ in adhesion values is what provided a lead to study the effects of chain length on the adhesion (plot not shown). In the case of the MCF7 cell line, the plot shows a line that has been plotted assuming that the value for CSC may be higher. With that exception, linearity is fairly clear.

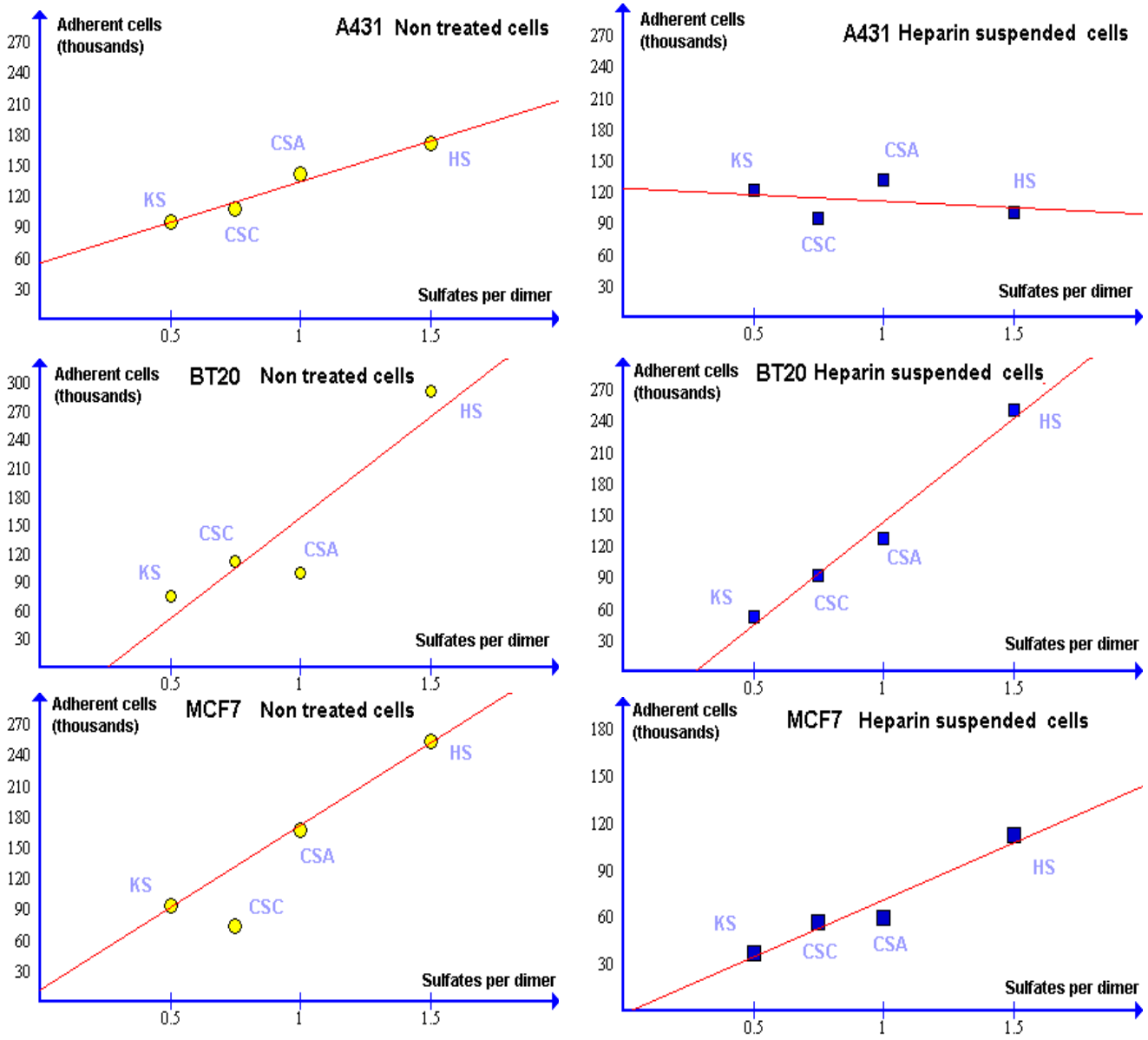

Figure 1. Adhesion *vs* charge (sulfate) per dimer graph for A431, BT20 and MCF7 non treated cells (left panel) and adhesion *vs* charge (sulfate) per dimer graph for A431, BT20 and MCF7 heparin suspended cells (right panel)**.** For each experimental group, the results are expressed as the mean percentage (+/- SD) of bound tumor cells in 9 cover slips.

Figure 1 (right panel) shows a plot of the adhesion *vs.* sulfate per dimer for MCF7 (bottom) , BT20 (middle) and A431 (top) of heparin suspended cells. Again, the similarity of the lines between cell lines and, more importantly, between different conditions – non treated and heparin resuspended – substantiates the assessment made before about the existence of this relationship. In the case of the A431 cell line, the plot shows a line with negative slope. It is possible that the value for KS may be distorting this plot, indicating that there may exist cell lines or conditions where the adhesion does not increase, or decreases, with polysaccharide charge. A modification of conditions may disrupt the tendency of regular cells to attach to polysaccharide chains depending on the charge density of the chain.

The y intercepts in these plots may have the following significance. If the y-intercept is not at zero level for zero charge (assuming that the extrapolation to zero can be performed), that would mean that adhesion levels do not drop to zero if the molecule is neutral or devoided of charges. This residual level then can be understood as the level of cell adhesion not associated with the specific

binding that provides the bulk of the adhesion numbers. If a mathematical relation can be deduced, this would predict the levels of adhesion of the homologous desulfated polysaccharide. If the y-intercept is at negative levels, that might imply that the cells require substrates with substantial charge density in order to be able to attach.

This discussion is clearly understood when looking at the plots, because they have been constructed using adhesion levels *versus* number of sulfate groups. If the graph is plotted using total charge per dimer, instead of sulfate groups only, the y-intercept may cross over the zero level (but not necessarily in all cases). In that case, the y-intercept will show the adhesion levels when the total number of sulfate groups is zero. As indicated, this second type of plot would be more useful in two cases: when plotting adhesion of naturally desulfated polysaccharides (i.e. hyaluronian) or when plotting adhesion levels of artificially desulfated GAGs.

4 CONCLUSIONS

The technique used in this and previous works have permitted the physical characterization of cancer cell and substrate interactions. In particular, the hypothesis that the adhesion of cells to polysaccharide chains has a linear dependence with the number of charges per disaccharide has been studied.

Results indicate that this relationship may be cell dependent and that usually takes the form of a linear increase when the number of charges (or sulfate groups) per disaccharide increases. These results are valid within the range of 0.5 to 2 sulfates (0.5 to 3 charges) per dimer. Extrapolation of values to zero charge per dimer – neutral polysaccharides – indicates that adhesion will not necessarily drop to zero levels when the molecule is neutral.

The purpose has been to show that there is a physical underlying mechanism dominating the adhesion of the cells, mainly the charge per dimer, that may operate even in changing cell environment or conditions, and that this mechanism can be studied and observed macroscopically working with whole cells not just using isolated molecular receptor. This in fact indicates that the cellular environmental conditions play a secondary role in the adhesion process and are unable – at least to the extent studied here – to substantially disrupt cellular adhesion.

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