Polymer Model of Cancer Cell Adhesion to Glycosaminoglycan Substrates Using the Radius of Gyration

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ABSTRACT: The hypothesis that the adhesion of whole cancer cells to glycosaminoglycan (GAG) substrates is a function of polysaccharide radius of gyration is presented. We use a worm-like chain (WLC) polymer model describing the global structure of the GAGs that will take into account the charge distribution and contour length of the polysaccharide implicitly in its radius of gyration to relate these parameters to cell adhesion. Specifically, we present measurements of the *in vitro* adhesion of cancer cells to isolated and individualized GAG substrates. We find that adhesion of the cells has a linear response with the radius of gyration and is essentially controlled by the charge per dimer. This dominating mechanism is not eliminated when the cells are subjected to resuspension in media with heparin. We then propose how these physical properties could be used to predict the preferred molecular structures of compounds for use as antimetastatic or antiinflammatory agents by comparing our results with known effective molecules. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 70–77, 2009

Key words: biopolymer; radius gyration; glycosaminoglycan; worm-like chain; cancer cell

INTRODUCTION

The study of the processes relating polymer physics and glycobiology will have increased interest in coming years, as recent events regarding modified heparin compounds have clearly demonstrated.[1] To contribute to the trend of polymer physicists working in applications of biopolymers,[2] here, we present a study that may prove useful for investigations in glycobiology with the use of polymer physics models.

Glycosaminoglycans (GAGs) are linear polysaccharides found in virtually all animal tissues, normally in covalent association with a protein backbone, forming proteoglycans. Most of the biological properties of proteoglycans are derived from the interactions of the GAG chains with their environment, hence the interest in developing physical models that could describe their interactions with whole cells. Additionally, sulfated polysaccharide and GAG involvement in tumor biology is well established[3,4] and appears to be related to the effect of the charge density of the biopolymer[5] as well as other factors, for instance, presence of L-iduronic acid[6] or length of the polymer backbone.[7,8] Essentially, the degree of sulfation and position of anionic groups within the polysaccharide chains affect the strength of the interaction between carbohydrate and polypeptides, giving them their varied biological functions. Early indications suggest that the degree of sulfation of a polysaccharide is what appears to have been the cause of the medical complications in the heparin case.[9]

These previous works prompted us to seek indications that there may be a functional dependence of the adhesion of cancer cells with the number of charges per dimer of the polyelectrolyte to which they are adhering. Using four different GAGs, [keratan sulfate (KS), heparan sulfate (HS), and chondroitin sulfate A (CSA) and C (CSC)] we prepared continuously coated glass GAG surfaces[10] to study the *in vitro* adhesion of three human cancer cell lines exhibiting different metastatic activities. Stromal therapy has emerged in recent years as a new strategy for cancer treatments. A possibility in the stromal therapy strategy for cancer treatments is the use of antiadhesive molecules to block adhesion of cancer cells. It has been reported that nonanticoagulant

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TABLE I
Model Equations, Parameters, and Assumptions

Radius of gyration	$R_g = (\langle r^2 \rangle / 6)^{1/2}$
Mean square end-to-end distance	$\langle r^2 \rangle_{\mathrm{WLC}} = 2L_pL_c\,(1 - (L_p/L_c) + (L_p/L_c)\,e^{-Lc/Lp})$
	For CSA, CSC, KS, assumed semiflexible charged chains $\rightarrow \langle r^2 \rangle_{\mathrm{WLC}} = 2L_pL_c$
	For HS, assumed short, rod-like, stiff chain, highly charged $\rightarrow \langle r^2 \rangle_{\mathrm{WLC}} = L_c^2$
Persistence length	$L_p = L_p^0 + L_p^{\mathrm{elect}} = L_p^0 + 0.32\,(l_B/l)\,f^2\kappa^{-1}$
Bare persistence length	$L_p^0 = 0.22$ nm
Debye length	$\kappa^{-1} = 0.8$ nm
Bjerrum length	$l_B = 0.7$ nm

When necessary input values for certain parameters are taken from the literature. GAGs are assumed linear chains without excluded volume effects, fully ionized in physiological solution and under equilibrium conditions not subjected to large stretching forces, so the enthalpic term is negligible. In these conditions, the persistence length and the Kuhn length are related by $L_K = 2L_p$.

species of heparin and polysulfated polysaccharides reduce the incidence of metastasis, up to 90% in some cases[11] and for this reason have been introduced as an antimetastatic therapeutic agent, for instance with the use of low-weight heparins.[12] With this application as motivation, we performed additional experiments designed to determine the effect of heparin on cell adhesion to the GAGs substrates, and the results were presented elsewhere.[13] Both untreated cells and cells that had been resuspended in heparin containing media were used, and facilitated an analysis of the adhesion relating the electronic charge per dimer of GAG and its chain length with adhesion numbers. In essence, our experiments gave indication that cancer cells may have a functional dependence of their adhesion 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 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, 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 included in our analysis is how the presence or absence of glucuronic acid and the sulfation of the glucosamine residue affect adhesion levels and is implicitly included in our central hypothesis.

GAGs consist of alternating uronic acid and hexosamine moieties typically found as side chains of a group of proteins known as proteoglycans. Chemically, these GAGs are primarily composed of the disaccharide repeat galactose and glucosamine -KS-; glucuronic acid and glucosamine -HS-; or glucuronic acid and galactosamine -CSA and CSC-. Thus, the species are differentiated by their monomer composition, the position, and configuration of their glycosidic linkages and the amount and location of their sulfate groups. On average, the number of charges per dimer in these polysaccharides can range from around 0.5 for KS to about four for heparin, taking into account sulfate and carboxylic groups.

A possible way to accommodate both molecular characteristics—electronic charge and length—into a single physical parameter is by using the R_g of the GAG polymer. It is widely accepted that most of the biological properties of proteoglycans are derived from their GAG content and structure, in the sense that the interaction with other molecules occurs through the polysaccharide chains, hence the interest in utilizing polymer physical models that could describe observed interactions between the GAG chains, devoid of the protein core, with whole cells. GAGs are polysaccharides that are considered as unbranched polymers and linear, highly sulfated and charged polyelectrolytes, with no or very low polydispersity. Consideration of GAGs as unbranched polymers permits the application of models with Gaussian chain distributions. We have applied the worm-like chain (WLC) model (with parameters summarized in Table I and described in Materials and Methods section) describing the values of R_g as a function of two parameters: contour length L_c and electronic charge f corresponding to the charge per disaccharide. Measurements of the relative stiffness of the molecules and their resulting R_g is biologically relevant in several situations not only in relation to cell adhesion but also, for instance, in relation to biosynthesis and transport of the polymer through the cells and tissues.[14] This biological relevance of R_g induced us to construct a model to relate the experimental data of the adhesion of the tumor cells with the R_g of the GAGs. As we show next, this type of analysis may be applicable to several biological processes where sulfated polysaccharides are involved, like cancer metastasis, and useful in predictive models for the development of therapeutic agents in glycobiology.[15,16]

We emphasize that our model describes the adhesion of cells by using a polymer model that takes

into consideration the global structure and charge density of the GAGs instead of its fine structure. Normal models indicate that cell and protein binding to polysaccharides is dependent on specific highly charged sequences within the polysaccharide structures, as is well known in the case of the anticoagulant activity of heparin, and to our knowledge, there are no models describing the possible effect of the global structure of the polysaccharides. Our intention is to show that the global structure of the GAG also plays a role in the biological effects of the biopolymer that is not in opposition to the localized charge sequences models.

MATERIALS AND METHODS

Description of the model

The WLC[17,18] model describes the polymer as a curved, continuous string of irregular shape but which remains linear in the range of a length known as persistence length, L_p. The contour length is given by $L_c = nl$, where n is the number of segments (in our case, disaccharides) of length l. Application of the WLC model to polysaccharides has already been performed previously.[19,20]

We apply the WLC model describing the values of R_g as a function of two parameters: contour length L_c and electronic charge, f corresponding to the charge per disaccharide. We use this expression for R_g because the attachment of the GAGs to the surface was at low density,[21] in the "mushroom" regime, where the coil dimension is similar to the unattached chains in its end-free state. Experimental values of R_g for GAGs have been given for hyaluronic acid,[22–24] but for sulfated polysaccharides there is only one known reference for chondroitin sulfates 4 and 6.[25]

The radius of gyration is given by $R_g = (\langle r^2 \rangle / 6)^{1/2}$, where $\langle r^2 \rangle$ is the mean square end-to-end distance of the molecule. This definition applies to all types of chains, ideal or real. We also assume that GAGs are linear chains without excluded volume effects due to the shortness of the chains. What distinguishes between different chain statistical models is the value of $\langle r^2 \rangle$. For the WLC model, the mean square end-to-end distance is given by

$$\langle r^2 \rangle_{\mathrm{WLC}} = 2L_pL_c(1 - (L_p/L_c) + (L_p/L_c)\mathrm{e}^{L_c/L_p}) \quad (1)$$

where the only fitting parameter is the persistence length because the contour length is fixed with value $L_c = nl$, where l is the GAG disaccharide length and n the number of disaccharides on the chain. The calculation of R_g in this case is then reduced to the calculation of the persistence length of the GAG chain.

Originally, the models describing persistence lengths did not include specific terms to account for the effect of the ionic atmosphere surrounding the polymer backbone, like in the case of polyelectrolytes. Odijk[26] first, and Skolnick and Fixman[27] later, introduced the concept of electrostatic persistence length. Essentially, the conformational properties of a polymer chain that contains ionizable groups may be described using the Debye-Hückel potential, where electrostatic interactions in the media are screened—exponentially—with a length scale in the order of the Debye screening length κ^{-1}. The model for the persistence length proposed by Odijk, Skolnick, and Fixman (OSF) introduced a quadratic dependence of the electrostatic persistence length with the Debye screening length, and this dependence has been recently modified by Dobryinin.[28] The resulting model describes a semiflexible polyelectrolyte under the conditions of the WLC model, with a total persistence length given as

$$L_p = L_p^0 + L_p^{\mathrm{elect}} = L_p^0 + 0.32(l_B/l)f^2\kappa^{-1} \quad (2)$$

where L_p^0 is the bare persistence length (that of a similar polyelectrolyte without charged groups), L_p^{elect} is the electrostatic persistence length, f is the disaccharide charge, κ^{-1} is Debye length, (which represents the thickness of the ionic atmosphere or double layer surrounding the polymer), $l_{\mathbf{B}}$ is the Bjerrum length, (distance at which the Coulomb interaction between two elementary charges in a dielectric medium of dielectric constant ε is equal to the thermal energy k_BT) and l is length of the disaccharide. We should note that, although the electrostatic persistence length contribution is not constant and varies with ionic strength,[29] the experiments were all performed under cell culture physiological conditions of ionic strength of 0.15 M.

To apply the WLC model given by eqs. (1) and (2) to GAGs, input values for a certain number of parameters are necessary. These values were taken from the literature or calculated using data provided by the manufacturer of the products used. The assumptions that we have made in the model are discussed as follows:

- We assume GAGs are fully ionized in physiological solution. In addition, the adhesion experiments were performed under equilibrium conditions, as required for application of the WLC model when calculating conformational properties.
- Polysaccharides can have a very high-density charge but rarely surpassing four charges per dimer (i.e., heparin). Thus, the range of the charge per dimer used in our analysis includes

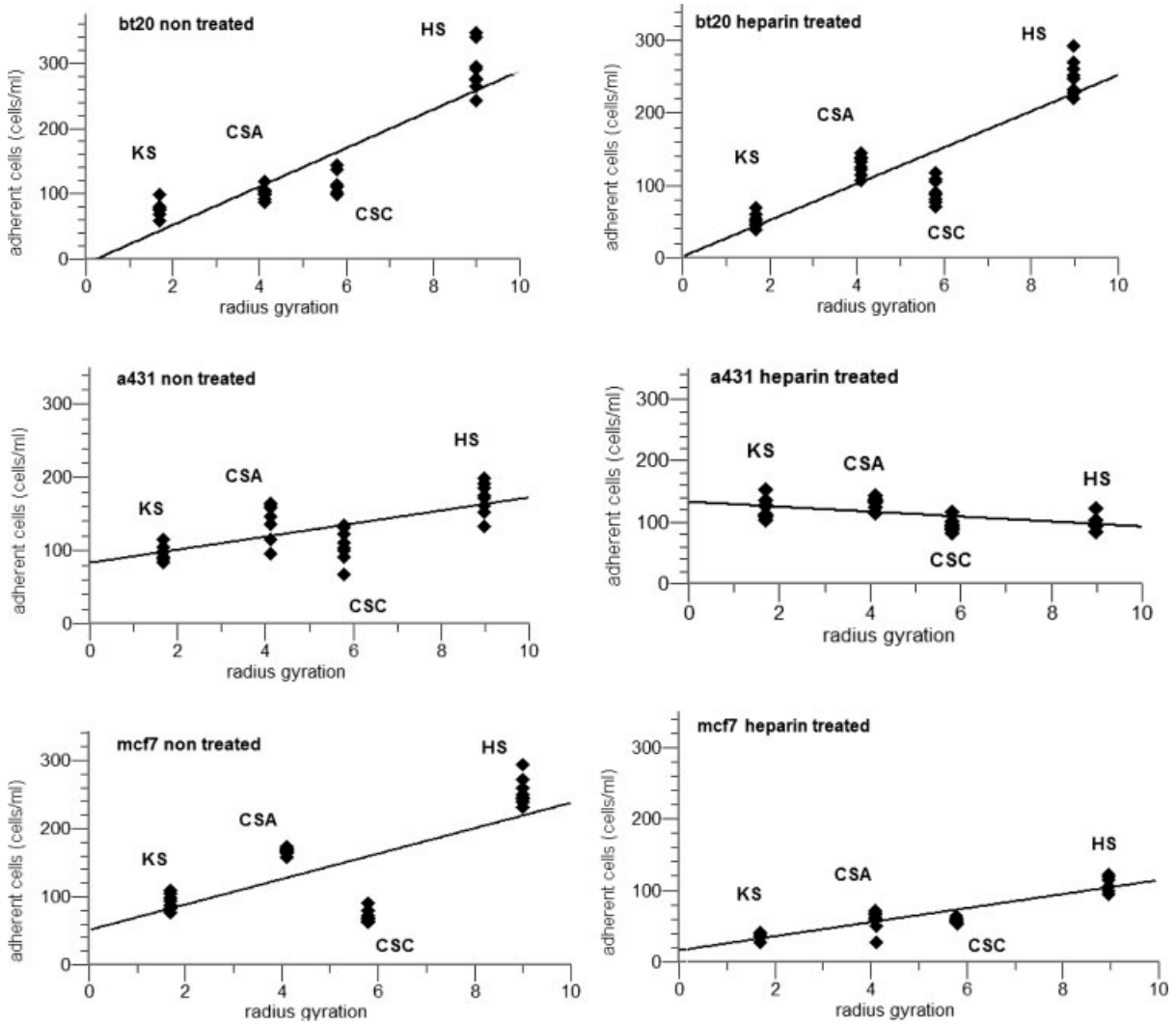

Figure 1 Plot of the adhesion *vs.* R_g for MCF7, BT20 and A431 of non-treated cells and heparin suspended cells. R_g is calculated using the WLC model for the glycosaminoglycans HS, CSA, CSC and KS. All tumor cell lines are human: BT20 (moderately metastatic) breast tumor cells, A431 (highly metastatic) epidermoid carcinoma cells and MCF7 and (non metastatic) breast tumor cells.

the major part of the spectrum of biological polyelectrolytes.

- The bare persistence length used is 0.22 nm, calculated by dividing by 2, the Kuhn length of dextran (0.44 nm),[30] a polysaccharide containing only D-glucose residues with an α(1,6) linkage. We use this value because no information is available for the persistence length of sulfated polysaccharides. We have used the same bare persistence length for all of our GAGs. Kuhn lengths for other non-sulfated polysaccharides can be found in Ref. 20. We note that the linearity of the results shown in Figure 1 will be lost if the same bare persistence lengths greater than 1 nm are applied to all GAGs in our study. However, the linearity of the results is maintained if we assume that the Kuhn lengths of our GAGs increase proportionally with the charge per dimer.
- The Debye length depends on both the polyelectrolyte and added salt concentration. In general, the Debye length, κ^{-1}, lies between the following values, 100 Å (10^{-3} Molar solutions) $< \kappa^{-1} <$ 3 Å (1 Molar solutions). The solutions used as cell culture medium provide an ionic strength of normal physiological values around 0.15 *M*. Using the equation given by Israelachvili,[31] the calculated value for κ^{-1} is then $\sim$ 0.8 nm. However, as discussed later, small variations in the Debye length have little effect on the results of the model.
- Bjerrum length is 0.7 nm for water at room temperature.
- HS is assumed to have a rod-like conformation due to its high charge per dimer and short length. Thus, the limit of the WLC for stiff chains, given by $\langle r^2 \rangle = L_c^2$ is used. This limit is

typically applied in polymer models to short chains.[32] On the other hand, CSA, CSC, and KS are assumed to be semiflexible charged chains, applying the WLC eq. (2).

- The number of dimers per chain are calculated by dividing the total mass of the GAG by the mass per dimer.
- The contour lengths L_c of the GAGs are estimated using values of the molecular mass of the products indicated by the manufacturers. Products used are HS (Seikagaku America, M.M. 11 kDa), KS (Seikagaku America, M.M. 13 kDa), CSC (Seikagaku America, M.M. 60 kDa), and CSA (Sigma Aldrich, M.M. 25 kDa). We note that we are applying our model to GAGs that are natural linear polymers with low polydispersity. The effects of polydispersity in the calculations in the model are very small and have little effect on the results of the model when other M.M. are used.
- Number of sulfates per disaccharide, charges per dimmer, and molecular mass of dimers are calculated following Lindahl and Hook.[33] 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, which is possible, is uncommon. Small deviations ($\sim$ 0.25 charges/dimer) of these values will not change the general trend that supports the hypothesis of linear dependence of adhesion with charge per dimer. Disaccharide monomer length l is averaged to 1 nm, using values from Squire et al.,[34] in line with data reported by Arnott and Scott[35] and Rees[36] that gave values between 0.92 and 1.16 nm.

RESULTS AND DISCUSSION

Contrarily to what it may appear, and to our knowledge, the parameters of interest for the physical characterization of GAGs, like radius of gyration or virial coefficients, among others, have been reported only partially—for hyaluronic acid, heparin, dermatan sulfate, and CSA—in the past, mainly during the decade of the 60s. There is no information regarding what would be the appropiate polymer model to use with sulfated polysaccharides. Given that they are unbranched polymers, it is possible to apply models with Gaussian chain distributions. For instance, one approach is to approximate the behavior of GAGs chains as ideal polymers in diluted physiological solution, with little coil overlap and commonly used models for biopolymers in solution, like the WLC. As polysaccharides, GAGs may be assumed to be more or less rigid chains of sequentially joined units by the C—O—C glycosidic bond that could give them the option to form helical structures under certain conditions. A qualitative description (see Rees, Ref. 37) depict them as extended and ribbon like, a description that fit skeletal (unbranched) polysaccharides in opposition to network (branched) polysaccharides. That means that random coil dimensions will be small for GAGs and then that in aqueous solutions they can be more accurately described by the WLC model.

We believe that our assumption of a rod-like conformation for HS and a semiflexible charge chains for CSA, CSC, and KS is well sustained by different factors. It has been noted that short polymers more closely resemble rods than flexible or WLCs. The radius of gyration is almost independent of solvent quality for low-molecular masses because short chains cannot exert long-range interactions. The effect of the charge density in the shape of the GAGs is that highly charged molecules like HS will be more rigid and rod-like, whereas KS or CSA will be more random-coiled molecules, but the general shape is a mix of charge density and intrinsic stiffness.

Applying the equations shown in Table I using the parameters and values shown on the left part of the Table II. We obtain the persistence length, the mean square end-to-end distance, and the radius of gyration for our GAGs, indicated on right part of the Table II. Table II shows that R_g increases with the square of the charge per dimer, making the charge of greater importance that the contour length. For instance, although the length of CSA is 1.5 times that of KS, the radius is only 2.5 times larger. The Debye length of 0.8 nm was calculated assuming physiological conditions as mentioned earlier. Equation (2) shows a κ^{-1} dependence of L_p. It has been noted that electrostatic persistence lengths may also vary with a κ^{-2} dependence. If the calculations are performed using κ^{-2} (0.64 nm), then the values of L_p and R_g also change. When a Debye length smaller or bigger—for instance 0.5, 0.64, or 1.5 nm—are used, small changes in R_g result, but the proportionality observed is maintained as with the calculations using 0.8 nm. This indicates that, albeit the effect of increased salt presence certainly may have an impact on R_g, the relationship between R_g of different GAGs does not change when the Debye length is varied. Thus, the linearity observed in the plots of the adhesion versus R_g will not be affected by charge screening.

The persistence length for HS is always the highest, making it stiffer or more rodlike, hence its treatment using the limit of the WLC model for stiff

TABLE II
GAG characteristic Data and Calculated Values of the Radius of Gyration

GAG	GAG characteristic data						Calculated model parameters (nm)		
	M.M. (kDa)	Dimer M.M. (Da.)	N	L_c (nm)	f	Sulf. groups	L_p	$\langle r^2 \rangle$	R_g
Keratan sulfate (KS)	13	403	32	32	0.50	0.50	0.264	16.94	1.68
Chondroitin sulfate C (CSC)	60	456	131	131	1.75	0.75	0.936	101.17	4.10
Chondroitin sulfate A (CSA)	25	456	54	54	2.0	1.0	0.738	201.42	5.79
Heparan sulfate (HS)	11	496	22	22	2.5	1.5	1.340	484.0	8.98

With application of equations from Table 1 combined with GAG characteristic data, we obtain the persistence length L_p, the mean square end-to-end distance $<r^2>$, and the radius of gyration R_g. M.M. is molecular mass in kiloDaltons (kDa) for the whole chain or Daltons (Da) for the dimer; n is number of dimers per chain; L_c is contour length; f is charge per dimer and last column indicates number of sulfate groups per dimer.

chains. R_g for HS is high enough to make HS appear as a rod to increase the chance that receptors on the cell surface encounter appropriate disaccharide sequences leading to an increased adhesion. In this sense, our model with the radius of gyration, although describing the molecule as a whole, implicitly takes into account the importance of the specific disaccharides sequences that permit increased interaction between HS and its receptors. On the contrary, low R_g values, for instance KS treated as semiflexible chain, exhibit reduced adhesion rates. The absence of a hexuronic acid in KS, which results in reduced charge density, may be one reason for the observed reduction in adhesion levels giving KS antiadhesive properties. The results of our model fit well with this antiadhesive properties[37] of KS and could explain why proteins bind proteoglycans on the cell surface (i.e., syndecans) using HS, but not KS, residues.[38]

Figure 1 shows plots of the adhesion versus R_g for MCF7, BT20, and A431 of nontreated cells and heparin suspended cells. There appears to be a linear relationship between the adhesion of the whole cells, and the calculated R_g of the GAG, which does not change when cells are resuspended in heparin prior to the adhesion experiment, whereas the effect of heparin in all cases is to reduce the adhesion and the slope of the plots. This reduction is tempered for the highly metastatic cell line (A431) and more profound for the nonmetastatic cell line (MCF7). It can be observed that adhesion levels generally are higher for HS and lower for KS with intermediate values for CSA and CSC. It appears then that high levels of sulfation (SO_3^- groups) provide the necessary charges to increase adhesion. This is shown by the fact that when the adhesion levels are plotted against the number of sulfate groups, instead of the radius of gyration, the plots keep the same linear relation, but increase their slopes (see Ref. 14).

Observation of the y intercepts in the plots in Figure 1 suggests that they may have the following significance. The extrapolation to zero R_g is equivalent to extrapolation to zero charge per dimer. If the y intercept is not at zero level for zero R_g (which is equivalent to zero charge per dimer) this indicates that adhesion levels do not drop to zero if the molecule is electrostatically neutral. This residual adhesion then could be understood as the level of cell adhesion not associated with the specific electrostatic binding, which provides the bulk of the adhesion numbers. This result could predict the levels of adhesion of the homologous desulfated polysaccharide for CSC, CSA, and HS molecules or homologous totally uncharged KS molecules. If the y intercept is at negative levels that could mean that the cells would require substrates with substantial charge density to be able to attach. An additional inspection of the plots indicates that the slopes are different for each cell line, treated or nontreated with heparin. However, for each cell line, the slope shows a decrease when the cells are treated with heparin with maximum effect for the A431 cell line resulting in a negative slope. This effect may be used as an indication that the reduction of binding events between cells and GAGs is more acute with increased values of the R_g. Biologically, the decrease of the slope with heparin treatment indicates that heparin would be more effective in reducing adhesion to high R_g molecules. In any case, as we have mentioned, the slope is clearly cell line dependent.

We note that our analysis has been performed in the so-called "mushroom regime" for GAG density on the substrates. We have concluded that when the R_g increases and the polymer is stiffer, as in the case of HS, the chain is able to expose, on average, a higher number of disaccharide sequences, permitting interactions for attachment with the receptors in the cell surface. We hypothesize that, in the transition to a "brush regime" where the molecules are stretched away from the surface due to electrostatic interactions of side chain groups, the increase in the GAG density would decrease the adhesion of the cancer cells, in absolute terms, for all GAGs, although this analysis is complicated by the scarcity of

information of the glycocalyx structure, chain orientation and molecular density of GAGs in the glycocalyx[39] and by further modifications in the expression of GAG in the ECM or cell surface in the areas surrounding primary tumors.

Applicability of the model

The analysis we have presented may be useful in the study of several biological processes in which GAGs are known to be involved, particularly in cell adhesion and cancer biology. Our approach may prove useful as an indicator of preferential metastatic targets, using information regarding GAG presence and its characteristics in different organs and tissues.[40] It has been proposed that the receptors that line the capillaries in endothelial cells are organ specific.[41,42] In this sense, differential expression of GAG molecules in the microvasculature and ECM of different organs (i.e., the heterogeneity of the GAGs in organs and tissues, which also depends on age[43] or malignancy[44]) may lead to different adhesion levels for metastatic cells. This suggests that altered GAG content and sulfation patterns are critical on the surface of either endothelial or cancer cells, for the promotion of angiogenesis and metastasis.[45]

Our approach should also be useful in the development of synthetic polysaccharides that work as antiinflammatory, antiangiogenic or antimetastatic agents.[46–48] It is known that sulfated polysaccharides inhibit metastasis and this is in fact the basis of the use of nonanticoagulant species of heparin and polysulfated polysaccharides as antimetastatic agents. It also has been reported that the most effective heparins are low-molecular weight heparins. It appears that heparins or sulfated polysaccharides may compete for available interaction sites with HS type molecules on the surface of cancer cells, thus possibly driving down the adhesion of metastatic cells in tissues. From our results, we can hypothesize that the most effective sulfated polysaccharides and heparins for these purposes would be described as being stiff rods, requiring (1) a medium to short number of units or disaccharides (low molecular weights), (2) presence of iduronic or glucuronic acid, and (3) high levels of sulfation, particularly 6-O sulfation—a description that fits very well, in broad terms, low-molecular weight heparins. Thus, our analysis would have predicted correctly the preferred molecular forms for these antimetastatic agents.

CONCLUSIONS

We recognize that several additional studies can be performed with other polysaccharides as well as additional analysis with the same GAG to take into account other parameters that may affect cell adhesion. For instance, experiments with the use of hyaluronan, which contains an acidic moiety but no sulfate group, can provide an idea of the contribution of the carboxylic group by itself and observe the effect on the adhesion when no sulfate groups are present. It would be interesting also to compare the differential effect of iduronic acid versus glucuronic acid. To assess the effect of higher charge density in the sequence and effect of iduronic acid, heparin or dermatan sulfate can also be tested. Evaluating the same GAG in a series with different degrees of polymerization and having various charge density values can also be tested. In this regard, it should be noted that no experiments have been performed to compare adhesion levels between molecules showing different sulfation location, for instance between 2 and 6 sulfated HSs. It is interesting to note, however, the case of CSA and CSC. CSA is mainly composed of four sulfated dimmers, whereas CSC is mainly composed of six sulfated dimers and both have similar charge density per dimer. However, the difference in chain length impedes any conclusion about the effect of 4 and 6 sulfation on adhesion levels.

Using the WLC model for polymer chains to calculate the radius of gyration of GAGs deposited on glass substrates, we found that the adhesion of the cells has a linear relationship with the radius of gyration of the GAG, and this relationship is primarily dependent on the charge per dimer of the polysaccharide, with a possible secondary contributing effect due to the contour length. We have also shown that this dominating mechanism is not eliminated when the cancer cells are subjected to heparin treatment. We propose that there is a physical underlying mechanism dominating the *in vitro* adhesion of the cancer cells, mainly the charge per dimer, that works independently of the two conditions tested and that this mechanism can be studied and observed macroscopically using whole cells *in vitro*, without having to isolate the molecules that act as GAG receptors on the surface of the cancer cells. We propose that the analysis of the radius of gyration may be useful in the design and development of the synthetic polysaccharides that work as antimetastatic agents.

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