Glycan and glycosaminoglycan binding properties of stromal cell-derived factor (SDF)-1α

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Introduction

Chemokines are 8–10 kDa cytokines that attract and activate leukocytes. They are divided into two major classes: α chemokines, also known as CXC chemokines due to the spacing of two conserved Cys residues by a single amino acid (aa), and β chemokines that present two adjacent conserved Cys (Murphy, 1994; Premack and Schall, 1996; Baggiolini et al., 1997). Chemokines are involved in migration of leukocytes to inflammatory sites through a regulated sequence of events that includes selectin-mediated rolling, attachment to endothelium, integrin-mediated arrest, and directional cues for migration provided by chemoattractants (Premack and Schall, 1996). Some chemokine receptors act also as coreceptors of human immunodeficiency virus (HIV) (D’Souza and Harden, 1996).

Indeed, if CD4 is HIV primary receptor (D’Souza and Harden, 1996), the preferential tropism of different HIV strains for either macrophages or transformed CD4+ cell lines corresponds to their use of different coreceptors (Feng et al., 1996; Premack and Schall, 1996; Baggiolini et al., 1997). The major coreceptor of cell line-adapted strains is CXCR4 (Feng et al., 1996; Oberlin et al., 1996), the physiological ligand of which is a chemokine stroma-derived factor (SDF)-1α (Bleul et al., 1996; Oberlin et al., 1996), a chemoattractant for monocytes and lymphocytes (Campbell et al., 1998) that induces arrest of lymphocytes rolling and stimulates calcium influx into cells (Campbell et al., 1998). SDF-1α inhibits infection of T cells by HIV isolates that use CXCR4 (Bleul et al., 1996; Oberlin et al., 1996), which are now termed X4 strains (Burger et al., 1998). CXCR4, also present on macrophages (Yi et al., 1998), is not used for entry by R5 strains that use CCR5, the receptor of β chemokines RANTES (Regulated on Activation, Normal T cell Expressed and Secreted), Macrophage Inflammatory Protein (MIP)-1α and MIP-1β (Alkhathib et al., 1996; Dragic et al., 1996). Moreover, no other mammalian chemokine has been shown to compete with SDF-1α for binding to CXCR4 receptor. It has been demonstrated that some chemokines bind to glycosaminoglycans (GAGs) (Kooppman and Krangel, 1997) and a GAG-binding site has been identified in MIP-1α; however, binding to GAGs is not a prerequisite step neither for receptor binding nor for signaling in vitro (Kooppman and Krangel, 1997). The gp120 of X4 strain HIV-1LAI specifically interacts also with sulfated polysaccharides, including heparin (Mbemba et al., 1992, 1994; Rodriguez et al., 1995), and heparitinase treatment of CD4+ lymphoid cells inhibits infection by X4 strains (Patel et al., 1993). Moreover, enzyme digestion of heparan sulfate but not of chondroitin sulfate from the surface of CD26+PM1 cells, a human T cell line selected for high CD26 expression, rendered them resistant to the antiviral effects of RANTES and MIP-1β for R5 HIV strains (Oravecz et al., 1997).

We show here that cell surface glycosaminoglycans (GAGs) are involved in the binding of stromal cell-derived factor (SDF)-1α to CD4+ lymphoid CEM or monocytic U937 cells, inasmuch as pretreating the cells with heparitinase or chondroitinase inhibits SDF-1α binding by 40–41% and 31–35%, respectively. Soluble heparin or chondroitin sulfate/mannan partially but significantly inhibits SDF-1α binding to the cells by 30–33% and 35–53%, respectively, while dextran has no significant effect. Taken together, these results indicate the role of GAGs in SDF-1α attachment to the cells. However, the effects of heparitinase and chondroitinase as well as those of heparin and chondroitin sulfate are not additive, which suggests that SDF-1α may attach to the cells through different GAGs, and also through other ligands. Soluble mannan also inhibits SDF-1α binding to the cells by 30–33%. Additivity between this effect and that of heparin or chondroitin sulfate is observed. Therefore, beside GAGs, mannose-containing species may also be involved in SDF-1α attachment to the cells. Accordingly, SDF-1α specifically binds to heparin-agarose and mannos-divinylsulfone agarose affinity matrices, and these interactions are inhibited respectively by soluble heparin, chondroitin sulfate, and mannan. We have previously shown that gp120 of X4 strain HIV-1LAI presents specific carbohydrate-binding properties for mannosylated derivatives, including mannan, and for GAGs including heparin. The present data therefore indicate that, in the same manner as HIV-1 Env, SDF-1α can interact with GAGs and glycans at the cell surface.

Key words: glycan/proteoglycan/SDF/heparin/chondroitin sulfate/mannan

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The aim of this study was to test whether SDF-1α, like gp120 of X4 strain HIV-1LA1, can interact with GAGs and glycans of lymphoid or monocytic cells expressing CD4 and CXCR4.

Results

Cells surface proteoglycans mediate SDF-1α binding to U937 and CEM cells

We examined whether cell surface proteoglycans were involved in SDF-1α binding to U937 and CEM cells, both known to express CD4 and CXCR4 (E.Mbemba et al., unpublished observations). Binding experiments of SDF-1α to cells pretreated or not with heparitinase or chondroitinase showed that SDF-1α bound in a dose-dependent and specific manner to both cell types since 10- to 300-fold excess of unlabeled SDF-1α significantly competed with [125I]SDF-1α binding (Figure 1, Tables I, II, and data not shown). The fact that heat-denaturation of SDF-1α significantly inhibited its binding to the cells (Figure 1, Tables I, II) further demonstrates the specificity of the interactions. This result also demonstrates the involvement of SDF-1α three-dimensional structure in its interactions with intact U937 and CEM cells. Because 300-fold excess of unlabeled SDF-1α significantly inhibited by 67–76% [125I]SDF-1α binding to the cells while heat-denaturation of the chemokine significantly inhibited these binding by 80–88% (Figure 1, Tables I, II), nonspecific binding of [125I]SDF-1α was considered, here, as the total bound radioactivity by the cells incubated in the presence of heat-denatured [125I]SDF-1α.

Pretreatment of the cells by heparitinase or chondroitinase, which digest heparan sulfate and chondroitin sulfate, significantly but partially inhibited SDF-1α binding to the cells by respectively 40–41% and 31–35%, as compared to control cells preincubated under the same conditions but in the absence of enzyme (Table I). Similar results of SDF-1α binding were observed when heparitinase or chondroitinase treatment was performed in the presence or the absence of protease inhibitors (data not shown); this rules out that reduced SDF-1α binding to the cells, after these enzyme treatment, is related to protease activity. Nevertheless, digestion of the cells with both, heparitinase and chondroitinase, did not modify SDF-1α binding, as compared to the results observed in the presence of each enzyme, separately (data not shown). To verify the efficiency of the enzyme-treatments, cells were submitted to a metabolic labeling by culture for 24 h in medium supplemented with [35S]O4 according to Uhlin-Hansen et al. (1993). In these conditions, heparitinase or chondroitinase treatment of the cells induced a significant release of [35S]-labeled GAGs, as assessed by the radioactivity detected in the supernatants of the cells: 7000–7300 c.p.m. and 5200–6300 c.p.m. were respectively detected in the supernatants of 10^6 U937 or CEM cells treated with heparitinase or chondroitinase, in the presence of protease inhibitors; while only 2400–3000 c.p.m. were detected in the supernatants of the cells incubated in buffer devoid of enzymes, which may be related in these latter conditions, with the spontaneous release in the medium of [35S]-labeled GAGs, as previously reported for human macrophages by Uhlin-Hansen et al. (1993).

Taken together, our data indicate that some steps of SDF-1α attachment to the cells may occur not only through different cell surface GAGs, heparan sulfate and chondroitin sulfate, but also through other ligands.

Preincubating SDF-1α at 37°C with heparin or chondroitin sulfate also partially, but significantly, inhibited SDF-1α binding to the cells in a dose-dependent manner, and this was also found using mannan as competitor, whereas dextran had...
Table I. Effect of heparitinase and chondroitinase treatment on [125I]SDF-1α binding to U937 and CEM cells

<table>
<thead>
<tr>
<th></th>
<th>U937 cells</th>
<th>CEM cells</th>
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<tbody>
<tr>
<td></td>
<td>Total bound radioactivity (c.p.m.)</td>
<td>% Inhibition of specific binding</td>
</tr>
<tr>
<td>Control</td>
<td>9415 ± 3800</td>
<td>7740 ± 3200</td>
</tr>
<tr>
<td>Heparitinase</td>
<td>5680 ± 1900***</td>
<td>40 ± 16</td>
</tr>
<tr>
<td>Chondroitinase</td>
<td>6106 ± 2060***</td>
<td>35 ± 10</td>
</tr>
<tr>
<td>Unlabeled SDF-1α</td>
<td>2000 ± 700***</td>
<td>76 ± 8</td>
</tr>
<tr>
<td>Heat-denatured SDF-1α</td>
<td>1730 ± 480**</td>
<td>81 ± 7</td>
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Table II. Effect of heparin, dextran, mannan, or chondroitin sulfate on [125I]SDF-1α binding to U937 and CEM cells

<table>
<thead>
<tr>
<th></th>
<th>U937 cells</th>
<th>CEM cells</th>
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<tr>
<td></td>
<td>Total bound radioactivity (c.p.m.)</td>
<td>% Inhibition of specific binding</td>
</tr>
<tr>
<td>Control</td>
<td>5900 ± 2120</td>
<td>6250 ± 1400</td>
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<tr>
<td>Heparin</td>
<td>3250 ± 1200*</td>
<td>45 ± 9</td>
</tr>
<tr>
<td>Dextran</td>
<td>5070 ± 2100</td>
<td>14 ± 10</td>
</tr>
<tr>
<td>Mannan</td>
<td>3920 ± 295**</td>
<td>33 ± 14</td>
</tr>
<tr>
<td>Chondroitin sulfate</td>
<td>3410 ± 1310**</td>
<td>42 ± 25</td>
</tr>
<tr>
<td>Unlabeled SDF-1α</td>
<td>1770 ± 390*</td>
<td>70 ± 9</td>
</tr>
<tr>
<td>Heat-denatured SDF-1α</td>
<td>710 ± 110*</td>
<td>84 ± 8</td>
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</tbody>
</table>

Enzyme-treated or untreated cells were incubated with 3 nM (50000 c.p.m.) of native or heat-denatured (10 min at 100°C) [125I]SDF-1α for 2 h at 4°C in RPMI-BSA, in the presence or not of 300-fold excess of unlabeled SDF-1α. Results are presented as means ± SD of 3–14 independent experiments performed in duplicate. The statistical significance of differences relative to controls was determined by the paired Student’s t test: *, P <0.01; **, P <0.02; ***, P <0.001.

Carbohydrate binding properties of SDF

Binding of SDF-1α to heparin-agarose or to mannose-divinylsulfone-agarose

According to these findings, we next examined whether SDF-1α interacted with heparin or mannose presented by affinity matrices. [125I]SDF-1α bound to heparin-agarose and mannose-divinylsulfone-agarose in a dose-dependent manner (Figure 2a, 3a), with a maximum corresponding to 50% of [125I]SDF-1α added, for heparin-agarose and 35% for mannose-divinylsulfone-agarose. These bindings were significantly inhibited by unlabeled SDF-1α, which demonstrated their specificity (Figure 2a, 3a, Tables III, IV). That [125I]SDF-1α binding to the two matrices was significantly inhibited by heat-denaturation of the chemokine or by its incubation with polyclonal anti-SDF-1 antibodies, but not with the isotype control, further demonstrates the specificity of SDF-1α interactions with heparin or mannose and the involvement of SDF-1α three dimensional structure in the interactions (Figure 2a,e, 3a,e, Tables III, IV). 300-fold excess of unlabeled SDF-1α significantly inhibited by 47–62% [125I]SDF-1α binding to the affinity matrices while heat-denaturation of the chemokine or its incubation with polyclonal anti-SDF-1 antibodies, significantly inhibited these binding by 77–85% (Figure 2a,e, 3a,e, Tables III, IV). Therefore, nonspecific binding of [125I]SDF-1α to both matrices was considered, here, as the total bound

no significant effect (Table II; data not shown), which confirms the specificity of these interactions (Table II). Percentages of maximum inhibition of SDF-1α bindings to the cells were, respectively, 45–52% and 42–56% in the presence of heparin or chondroitin sulfate. In addition, soluble mannan also inhibited by 30–33% SDF-1α binding, strongly suggesting that mannose-containing species can also be involved in some steps of SDF-1α attachment to the cells.

Incubation of SDF-1α with a mixture of soluble heparin and chondroitin sulfate did not modify its binding to the cells, as compared with the results observed in the presence each compound, separately (data not shown). However, incubation of SDF-1α with a mixture of heparin and mannan inhibited by 87 ± 8% and 85 ± 3% (n = 3) its bindings to the U937 and CEM cells respectively, while incubating it with a mixture of chondroitin sulfate and mannan inhibited these bindings by 78 ± 4% and 58 ± 4% (n = 3), respectively. Therefore, the effects of mannan and those of heparin or chondroitin sulfate are additive, in most cases.

Taken together, our data indicate that surface GAGs, heparan sulfate or chondroitin sulfate, and possibly glycans, such as mannose-containing species, may be involved in SDF-1α initial attachment to monocytic CXCR4+ U937 and lymphoid CXCR4+ CEM cells.
Fig. 2. Influence of physicochemical conditions on the binding of SDF-1α to heparin-agarose. (a) SDF-1α concentration: native (solid squares, open squares) or heat-denatured (open triangles) [125I]SDF-1α was incubated for 1 h at 37°C with 20 µl of matrix in Tris-Ca-BSA (pH 7.4) in the absence (solid squares) or the presence (open squares) of 300-fold excess of unlabeled SDF-1α. (b) Temperature: [125I]SDF-1α was incubated with 20 µl of heparin-agarose at 4°C, 20°C and 37°C. (c) Influence of pH: 3 nM [125I]SDF-1α were incubated with 20 µl of heparin-agarose at pH ranging from 4 to 8.2. (d) Influence of Ca²⁺ concentration: [125I]SDF-1α was incubated with 20 µl of heparin-agarose in Tris-BSA in the presence of 2.5 mM CaCl₂ or 10 mM EDTA. (e) [125I]SDF-1α (SDF-1) was preincubated or not for 1 h at 20°C with goat polyclonal anti-human SDF-1 (1.3 µg/ml; anti-SDF) or its isotype control (IgG). Results are presented as means ± SEM of three independent experiments performed in duplicate.

Fig. 3. Influence of physicochemical conditions on the binding of SDF-1α to mannose-divinylsulfone-agarose. (a) SDF-1α concentration: native (solid squares, open squares) or heat-denatured (open triangles) [125I]SDF-1α was incubated for 1 h at 37°C with 20 µl of matrix in Tris-Ca-BSA (pH 7.4) in the absence (solid squares) or the presence (open squares) of 300-fold excess of unlabeled SDF-1α. (b) Temperature: [125I]SDF-1α was incubated with 20 µl of heparin-agarose at 4°C, 20°C and 37°C. (c) Influence of pH: 3 nM [125I]SDF-1α were incubated with 20 µl of heparin-agarose at pH ranging from 4 to 7.4. (d) Influence of Ca²⁺ concentration: [125I]SDF-1α was incubated with 20 µl of mannose-divinylsulfone-agarose in Tris-BSA in the presence of 2.5 mM CaCl₂ or 10 mM EDTA. (e) [125I]SDF-1α (SDF-1) was preincubated or not for 1 h at 20°C with goat polyclonal anti-human SDF-1 (1.3 µg/ml; anti-SDF), or its isotype control (IgG). Results are presented as means ± SEM of three to four independent experiments performed in duplicate.
radioactivity by the matrices incubated in the presence of heat-denatured [¹²⁵I]SDF-1α.

Binding was only increased slightly at 37°C relative to 4°C and 20°C (Figure 2b, 3b). It was pH-dependent for heparin-agarose, decreasing from pH 4 to 4.6, increasing up to pH 7.8 then decreasing at pH 8.2; this indicates occurrence of electrostatic interactions (Figure 2c). It was pH independent for mannose-divinylsulfone-agarose, remaining constant from pH 4 until pH 7.4 (Figure 3c).

Bindings to heparin-agarose and to mannose-divinylsulfone-agarose were both Ca²⁺-independent because they were not prevented when EDTA was added before initiation of the reaction (Figures 2d, 3d). On contrary, a significant increase of SDF-1 binding to heparin-agarose was observed in the presence of 10 mM EDTA (Figure 2d). Binding of [¹²⁵I] SDF-1α (5 nM) to mannose-divinylsulfone-agarose in the presence of 2.5 mM of Cl₂Ca (15,650 ± 2500 c.p.m.; n = 4) did not significantly differ from that observed in the presence of 10 mM EDTA (16,700 ± 3000 c.p.m.; n = 4) (Figure 3d).

Binding of SDF-1α to heparin-agarose was specifically reversed by soluble heparin but not by dextran (Figure 4). Binding of SDF-1α to mannose-divinylsulfone-agarose was specifically reversed by mannan, heparin but not by dextran (Figure 4 and data not shown). As determined by SDS–PAGE, the molecules specifically eluted from the matrices had then the same 8 kDa MW as SDF-1α in the initial preparation.

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<th>Table III. Effect of heparin, chondroitin sulfate and dextran on [¹²⁵I]SDF-1α binding to heparin-agarose</th>
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<tr>
<td>Control</td>
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<tr>
<td>Heparin</td>
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<tr>
<td>Chondroitin sulfate</td>
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<td>Dextran</td>
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<tr>
<td>Heat-denatured SDF-1α</td>
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<td>Anti-SDF-1α</td>
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<td>Isotype control (goat IgG)</td>
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<td>Unlabeled SDF-1α</td>
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Native or heat-denatured [¹²⁵I]SDF-1α (3 nM; 50 000 c.p.m.) was incubated for 1 h at 37°C, in the presence or the absence of 300-fold excess of unlabeled SDF-1α, with 0–330 µg/ml heparin, chondroitin sulfate, or dextran, or with 1.3 µg/ml of polyclonal goat anti-SDF-1 or the isotype control, and the mixture was then added to the matrix. Concentrations for maximum inhibition in the presence of GAGs are 330 µg/ml. Results are presented as means ± SD of three to four independent experiments performed in duplicate. The statistical significance of differences relative to controls was determined by the paired Student’s t test: *, P <0.01; **, P<0.02; ***, P<0.05.

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<tr>
<th>Table IV. Effect of mannan and dextran on [¹²⁵I]SDF-1α binding to mannose-divinylsulfone-agarose</th>
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<td>Control</td>
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<td>Mannan</td>
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<tr>
<td>Dextran</td>
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<tr>
<td>Me-α-Man</td>
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<td>Me-α-Gal</td>
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<tr>
<td>Heat-denatured SDF-1α</td>
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<td>Anti-SDF-1α</td>
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<td>Isotype control (goat IgG)</td>
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<td>Unlabeled SDF-1α</td>
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Native or heat-denatured [¹²⁵I]SDF-1α (3 nM; 50 000 c.p.m.) was incubated for 1 h at 37°C in the presence or the absence of 300-fold excess of unlabeled SDF-1α, with mannan, dextran, Me-α-Man or Me-α-Gal, or with 1.3 µg/ml of polyclonal goat anti-SDF-1α or the isotype control, and the mixture was then added to the matrix. Results are presented as means ± SD of three to four independent experiments performed in duplicate. Concentrations for maximum inhibition are 330 µg/ml for mannan and 30 nM for Me-α-Man or Me-α-Gal. The statistical significance of differences relative to controls was determined by the paired Student’s t test: *, P < 0.05; **, P < 0.02; ***, P < 0.01. In addition, the significance of the difference between the binding in the presence of Me-α-Man or of Me-α-Gal was P < 0.05.
which demonstrates that they corresponded indeed to SDF-1α (Figure 4; data not shown).

In Tables III and IV are presented the mean 50% inhibiting concentrations (C50) and percentages of maximum inhibition obtained when heparin or mannure were incubated with SDF-1α before addition to heparin-agarose or to mannose-divinylsulfone-agarose, which significantly inhibited SDF-1α binding to the matrices in a dose-dependent manner (Tables III, IV; data not shown). Dextran had a limited but significant effect on SDF-1α binding to heparin-agarose, as compared to heparin (Table III), while chondroitin sulfate had an intermediate effect, which further indicates the specificity of SDF-1α interaction with heparin-agarose. Dextran had no effect on SDF-1α binding to mannose-divinylsulfone-agarose, while Me-α-Man had a limited but significant inhibitory effect (Table IV), slightly, but significantly higher than that observed with Me-α-Gal (Table IV).

Thus, SDF-1α specifically interacts with heparin-agarose and mannose-divinylsulfone-agarose.

Discussion

Here, we demonstrate the occurrence of specific, dose-dependent, and reversible interactions between SDF-1α and heparin or mannose presented by affinity matrices. The pH dependence of SDF-1 interactions with heparin-agarose indicates the involvement of electrostatic interactions, as expected from previous data observed with MIP-1α (Koopmann and Krangel, 1997) and in accordance with the fact that SDF-1α reveals a positively charged surface (Dealwis et al., 1998) ideal for binding to negatively charged structures. That SDF-1α binding to an heparin-agarose affinity matrix was inhibited by soluble heparin with a C50 of about 40 µg/ml, representing a value of 10⁻⁴ M disaccharide units, indicates that SDF-1α interacts specifically with this GAG, albeit with low affinity, in our experimental conditions. Chondroitin sulfate also inhibited SDF-1α binding to heparin-agarose, but to a lesser extent than heparin; that 300 µg/ml chondroitin sulfate are necessary to inhibit 50% of SDF-1α binding to heparin-agarose, indicates that this GAG also interacts with SDF-1α, but with about a 7-fold lower affinity than for heparin. However, these results do not rule out the occurrence of much higher affinity interactions between SDF-1α and more homogeneous preparations of heparin, or between SDF-1α and heparin sulfate proteoglycans presented at the cell surfaces. It is likely that specific monosaccharide sequences of heparan sulfate are required for high affinity binding to SDF-1α. Whether these interactions involved Arg residues in the SDF-1α(aa sequence as reported for MIP-1α (Koopmann and Krangel, 1997) is under investigation.

We also found that heparan sulfate or chondroitin sulfate mediated SDF-1α attachment to monocytic U937 and lymphoid CEM cells. This attachment was significantly, but partially, decreased by heparitinase or chondroitinase pretreatment of the cells. However, digestion of the cells with both, heparitinase and chondroitinase, did not modify SDF-1α binding, as compared to the results observed in the presence of each enzyme. These results suggest that SDF-1α may attach to the cells through different GAGs. Alternatively, these data also indicate that other SDF-1 ligands may be involved. Whether SDF-1 interactions with GAGs occur before or at the same time that its binding to its receptor, CXCR4, has to be determined (Feng et al., 1996; Oberlin et al., 1996). Whether the GAGs binding properties of SDF-1 are necessary for the interaction of the chemokine with its receptor has also to be elucidated. It has indeed been demonstrated that GAG-binding capability is not a prerequisite for the biological activity of MIP-1α (Koopmann and Krangel, 1997).

Heparitinase cleaves glucosamine (1–4)-glucuronic acid linkages, which is more common within heparan sulfate sequences than in heparin. It needs glucosamine N-acetylation or N-sulfation but tolerates only O-sulfation of C-6 in the amino sugar. Such structural features correspond especially to heparan sulfate (Linhardt et al., 1990). The heparan sulfate differs from the heparin group because it is more acetylated and less sulfated than heparin.

Preincubation of SDF-1α with heparin or chondroitin sulfate also significantly, but partially, inhibited its binding to the cells. Maximum inhibition of SDF-1α binding to the cells were respectively 45–52% and 42–56% in the presence of heparin or chondroitin sulfate. However, the effects of heparin and chondroitin sulfate were not additive.

Taken together, our data indicate that surface GAGs are involved in SDF-1α initial attachment to monocytic CXCR4+U937 and lymphoid CXCR4+CEM cells. In the same experimental conditions, heparin and chondroitin sulfate partially, but significantly, inhibit SDF-1α binding to human primary peripheral blood lymphocytes and monocyte-derived macrophages (L.Gattegno et al., unpublished observations). Moreover, specific binding of SDF-1α to primary cells such as human peripheral blood monocytes and T-lymphocytes has been demonstrated (Ueda et al., 1997). Furthermore, proteoglycans, sensitive to both chondroitinase and heparitinase,
have been detected on monocyte-derived macrophages surface membranes (Chang et al., 1998). In addition, it has been demonstrated that RANTES specifically binds, not only to heparan sulfate (Oravecz et al., 1997), but also to chondroitin sulfate (Amzazi et al., 1998; Wolf et al., 1999; L.Gattegno and Saffar, unpublished observations).

Our present data strongly indicate that SDF-1α, like RANTES, can bind to cell surface GAGs such as heparan sulfate and/or chondroitin sulfate, of different cell types (lymphoid and monocyte cell lines, primary lymphocytes, and macrophages).

Finally, we found that SDF-1α binding to mannosyl-divalent-sulfone-agarose was inhibited by mannan with a C50 of 40 µg/ml, which is indicative of low affinity interactions. Mannan also interfered with SDF-1α binding to CEM and U937 cells as demonstrated in the present study. The inhibitory effects of heparin and mannan and in some cases, of chondroitin sulfate and mannan, were additive. Moreover, we currently observe that mannan also partially, but significantly, interferes with SDF-1α binding to human primary peripheral blood lymphocytes and monocyte-derived macrophages (L.Gattegno and Saffar, unpublished data).

Taken together, our data strongly suggest that in addition to cell surface GAGs, some glycan structures such as manno-containing species, are also involved in some chemokine attachment step to cell lines and to primary cells.

We have previously shown that gp120 of X4 strain HIV-1LAI specifically interacts with N-Glycans of Concanavalin A-reactive CD4-free glycopeptides prepared from U937 cell membranes, and that these glycopeptides can inhibit HIV-1LAI infection of U937 cells as well as of CEM cells (Mbemba et al., 1996). Whether this ability of SDF-1α, which may thus be compared to that of gp120 of HIV-1LAI, to specifically bind GAGs and glycans is essential for CXCR4 binding or signaling as well as the physiological role of this bindings is presently under investigation.

At the present time, the role of GAGs association in the function of chemokines is not elucidated. Furthermore, the association between a chemokine and glycans such as mannan has not been previously demonstrated. Whether cell-surface proteoglycans and/or glycans are essential for the appropriate conformation and attachment of SDF-1 to the membrane of its target cells, has to be elucidated since glycoconjugates, glycoproteins, glycolipids, and proteoglycans are known to mediate fundamental cellular processes such as cell–cell, cell–matrix adhesion, motility, growth, and signaling (Sanderson et al., 1994; Nelson et al., 1995; Faham et al., 1996; Reichsman et al., 1996).

Proteoglycans consist of a protein core and one or more covalently attached GAGs chains, which consist of alternating residues of an amino sugar and an uronic acid. As the chains polymerize, they undergo various sulfation and epimerization reactions (Sanderson et al., 1994). Proteoglycans exhibit tremendous structural heterogeneity due, at least in part, to variation in the lengths of GAGs chains and in the extent and pattern of sulfation and epimerization (Lindhall, 1989; Lind et al., 1993). Determining if specific glycoforms have unique biological properties is therefore a major point. Accordingly, the low affinity interactions between SDF-1α and heparin observed in the present study do not rule out that much higher affinity interactions between this chemokine and cell-surface proteoglycans occur at different stages of the cells differentiation.

In the present study, binding experiments before and after removal of GAGs from the cell surface as well as competition experiments performed with heparin or chondroitin sulfate demonstrate involvement of GAGs in SDF-1α attachment to monocyte and lymphoid cells. Although HIV Env and chemokines such as SDF-1α bind to cell surface GAGs, questions about the nature of these interactions remain unanswered. Are specific oligosaccharide sequences required for these bindings? What is the role of GAGs-Env and GAGs-chemokine interactions in HIV infection? Answers to these questions may lead to polysaccharide-based antiviral pharmaceutical agents. In addition, as CXCR4 and SDF-1 are a specific receptor-ligand pair that acts in cardiac and blood cell development (Horuk, 1998), the roles of glycans and proteoglycans in these processes have also to be elucidated.

Materials and methods

Radiolabeling of SDF-1α

Synthetic SDF-1α (aa: KPVSLSYRCPRFFESHVARANVKH-LKILNTPNCALQIVA RLKNNRQVCDPKLKWIOYELE-KALN was a gift from F.Baleux, Institut Pasteur, Paris, France). Radiolabeling was performed with iodobeads (Pierce Europe, BA Oud-Beijerland, The Netherlands) according to the manufacturer’s instructions. Specific activity was about 0.2 MCi/µg. Its homogeneity was assessed by sodium dodecyl sulfate–polyacrylamide gel (16%) electrophoresis (SDS–PAGE).

Cells

CD4+CXCR4+ cells of the monocytic U937 and lymphoid CEM lines were cultured at 37°C in RPMI 1640, 10% heat-inactivated fetal calf serum (GIBCO-BRL, Paisley, Scotland).

Enzyme treatment

An amount of 5 × 105 cells was incubated for 1 h at 37°C in 50 µl PBS, 0.05% BSA, and 1 µM heparitinase (heparitin sulfate eliminase, EC.4.2.2.8) or chondroitinase (chondroitinase ABC eliminase, EC.4.2.2.4), both from Sigma (St. Louis, MO) supplemented or not with protease inhibitors (10 mM PMSF, 25 mM phenanthroline, 5 mM iodoacetamide, 100 µg/ml apro tin, all from Sigma). In some parallel experiments, cells were coincubated with both enzyme heparitinase and chondroitinase, or treated subsequently with each of these enzymes. The enzymes were not toxic for the cells, as assessed by trypan blue exclusion.

Metabolic labeling of the cells

Cells were incubated as described by Uhlin-Hansen et al. (1993) for 24 h in culture medium supplemented with 50 µCi/ml [35S]SO42−, carrier-free (Amersham, Little Chalfont, England). Cells were then incubated, as described above, with heparitinase or chondroitinase to release [35S]-labeled cell surface glycosaminoglycans. Supernatants were collected and measured with a β counter (Beckman, Paris, France).

SDF-1α binding to the cells

Cells (5 × 105) in 100 µl RPMI 1640, 0.05% BSA (RPMI-BSA) were incubated for 2 h at 4°C with 50 µl of native or
heat- treated (10 min at 100°C) [125I] SDF-1α (0–200 × 10^5 c.p.m.; 0–10 × 10^-9 M) in the presence or absence of 10- to 300-fold excess unlabeled SDF-1α. After two washes (700 × g, 10 min, 4°C), cell-bound radioactivity was measured with a γ counter (LKB, Paris, France). Nonspecific binding was determined in the presence of 300-fold excess unlabeled chemokine or in the presence of heat-denatured chemokine. The effect of heparitinase, chondroitinase, or both enzymes on SDF-1α (0.5 × 10^5 c.p.m.; 3 × 10^-9 M) binding to the cells was determined. In parallel, the effect of heparin (170 U/mg; sodium salt, from porcine intestinal mucosa), mannan (from Saccharomyces cerevisiae), chondroitin sulfate (70% chondroitin sulfate A; sodium salt from bovine trachea), or dextran (all up to 330 µg/ml; all from Sigma), or mix of these compounds, on SDF-1α binding to the cells was examined at constant concentrations that did not alter cell viability, as follows: [125I] SDF-1α in RPMI-BSA was incubated for 60 min at 37°C with different concentrations of heparin, chondroitin sulfate, mannan, or dextran, and the mixture was then added to the cells and incubated as described above. In some experiments, mixes of these compounds, were incubated with [125I] SDF-1α.

**Binding of SDF-1α to heparin-agarose or to mannoside-divinylsulfone-agarose**

Binding of [125I] SDF1α to heparin-agarose (Sigma) or to mannoside-divinylsulfone-agarose (E.Y. Laboratories, San Mateo, CA) was investigated as follows: 20 µl of affinity matrix were suspended in an equal volume of buffer 0.02 M Tris, 0.15 M NaCl, 0.0025 M CaCl2, 0.05% BSA, pH 7.4 (Tris-Ca-BSA). After incubation for 1 h at 37°C with 0 to 10^-8 M native or heat-treated (10 min at 100°C) [125I]SDF-1α in the absence or the presence of 10- to 300-fold excess of unlabeled SDF-1α, unbound SDF-1α was washed out twice in 500 µl of buffer. Solid phase-bound radioactivity was counted, and results were expressed as means of duplicates of at least three independent experiments. Nonspecific binding was determined in the presence of 300-fold excess unlabeled chemokine or in the presence of heat-denatured chemokine. The physicochemical characteristics of the interaction were analysed by performing the assays under different conditions: [125I] SDF-1α diluted in 50 µl of buffer was incubated with 20 µl affinity matrix in 0.02 M Tris, 0.15 M NaCl, 0.05% BSA buffer (Tris-BSA), pH 7.4, supplemented by 0.0025 M CaCl2 or 0.010 M disodium salt EDTA(Sigma); or the experiments were conducted in Tris-Ca-BSA at 4°C, 20°C, or 37°C, or at different pH ranging from 4 to 8.2. Reversal of SDF-1α binding to heparin-agarose or to mannoside-divinylsulfone-agarose was investigated as follows: 250 µl of [125I] SDF-1α (3 × 10^-4 M) were incubated for 1 h at 37°C with 250 µl of affinity matrices in Tris-Ca-BSA. Unbound [125I] SDF-1α was washed out thrice in 1 ml of buffer, and the matrix was incubated at 20°C for 5 h with gentle shaking in 250 µl buffer supplemented or not with heparin, mannan or dextran (5 mg/ml). The specifically reversed molecules were then analyzed by SDS–PAGE (16%).

In parallel, [125I] SDF-1α was preincubated for 45 min at 37°C with 0–330 µg/ml heparin, chondroitin sulfate, mannan, Me-α-Man, Me-α-Gal, or dextran (all from Sigma), before adding 20 µl of the affinity matrix. In some experiments, [125I] SDF-1α was preincubated for 1 h at 20°C with goat polyclonal anti-human SDF-1 (1.3 µg/ml; R & D Systems, UK), or its isotype control (R&D). Coincubation was then performed as above. Unbound [125I] SDF-1α was washed out and bound radioactivity was counted.

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**Abbreviations**

SDF, stromal cell-derived factor; gp, glycoprotein; GAG, glycosaminoglycan; HIV, human immunodeficiency virus; RANTES, regulated on activation, normal T cell expressed and secreted; MIP, macrophage inflammatory protein; C50, 50% inhibiting concentration.

**References**


Carbohydrate binding properties of SDF


