Heparan Sulfate Targets the HIV-1 Envelope Glycoprotein gp120 Coreceptor Binding Site*

Received for publication, January 25, 2005, and in revised form, March 18, 2005
Published, JBC Papers in Press, March 28, 2005, DOI 10.1074/jbc.M500911200

Romain R. Vive`s‡, Anne Imberty§, Quentin J. Sattentau¶, and Hugues Lortat-Jacob¶¶

From the ‡Institut de Biologie Structurale, CNRS-Commissariat à l’Energie Atomique-Université Joseph Fourier, UMR 5075, 41 Rue Horovitz, 38027 Grenoble, France, the §Centre de Recherches sur les Macromolécules Végétales-CNRS (affiliated with Université Joseph Fourier), 38041 Grenoble, France, and the ¶Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, United Kingdom

Human immunodeficiency virus (HIV) attachment to host cells is a multi-step process that involves interaction of the viral envelope glycoprotein (Env) with the primary receptor CD4 and coreceptors. HIV gp120 also binds to other cell surface components, including heparan sulfate (HS), a sulfated polysaccharide whose wide interactive properties are exploited by many pathogens for attachment and concentration at the cell surface. To analyze the structural features of gp120 binding to HS, we used soluble CD4 to constrain gp120 in a specific conformation. We first found that CD4 induced conformational change of gp120, dramatically increasing binding to HS. We then showed that HS binding interface on gp120 comprised, in addition to the well characterized V3 loop, a CD4-induced epitope. This epitope is efficiently targeted by nanomolar concentrations of size-defined heparin/HS-derived oligosaccharides. Because this domain of the protein also constitutes the binding site for the viral coreceptors, these results support an implication of HS at late stages of the virus-cell attachment process and suggest potential therapeutic applications.

Human immunodeficiency virus (HIV)1 adsorption and entry into potential target cells is initiated by the binding of the viral envelope glycoprotein (Env) to a set of cell surface molecules. This comprises both attachment receptors, including CD4 (1) and heparan sulfate (HS) (2), and entry receptors. The latter belong to the chemokine receptor family, among which CCR5 and CXCR4 are the most physiologically relevant (3). The surface envelope glycoprotein gp120 features all of the binding determinants for both attachment and entry receptors and constitutes the central element for all interactive events occurring during the pre-entry steps. Accordingly, this molecule is structurally complex and exhibits unusual features. It consists of a core structure organized into an inner domain, an outer domain, a bridging sheet spanning these two domains, which appears to be partially unfolded or disordered, and five surface-exposed variable loops, V1 to V5 (4). Binding to CD4 triggers extensive structural alterations in gp120. These include shifts of the V1/V2 and V3 loops and drive the exposure and/or conformational changes of a region within the bridging sheet called the CD4-induced (CD4i) epitope (5, 6). In conjunction with the V3 loop, this region makes up the binding site for chemokine-receptors (7, 8).

The V3 loop is also an essential determinant of HS recognition (9). HS is a sulfated polysaccharide of the glycosaminoglycan family characterized by wide interactive properties and critical functions in many biological processes (10). In particular, a large number of viruses interact productively with HS, a binding that facilitates attachment to host cells and subsequent infection (11).

It has been known for a long time that removal of cell surface HS or the use of soluble glycosaminoglycans, including heparin (HP) and HS as competing agents, reduced both HIV attachment and infection on several cell lines, including CD4-positive HeLa cells, macrophages, and T-cell lines (12–14). HS was thus thought to increase infectivity by favoring viral particle concentration at the cell surface. However, recent evidence has suggested that the role of HS in HIV infection goes well beyond that of a low specificity pre-attachment site. In macrophages, HS can compensate for a low level of CD4 expression during viral attachment and modulate HIV replication (14). HS expressed by CD4 negative brain endothelial cells has been shown to play a crucial role in HIV entry (15). In addition, it has been reported that cell surface HS preserved HIV infectivity for days, enhanced in trans infectivity, and provided conditions that boosted replication in T-cells (16).

Using surface plasmon resonance to analyze the binding of gp120 to HS, we reported that this interaction did not fit any simple binding model (17). This could be the result of the protein’s intrinsic flexibility or indicate the existence of multiple binding motifs. A clarification of gp120/HS binding determinants would thus help in understanding the role of this interaction during infection and provide insights into unexplored modes of therapeutic intervention. To further characterize the binding of gp120 to HS, we investigated whether CD4-induced conformational change in gp120 had any impact on its HS binding properties. This was performed on gp120 from the laboratory-adapted HIV-1 isolate HXBc2, the core structure, receptor binding, and interaction of which with neutralizing antibodies are similar to those of a primary clinical isolate (18). We found that gp120 in its CD4-bound state had substantially increased binding to HS as compared with free gp120 and demonstrated by experimental analysis and molecular modeling that the HS binding site on gp120 is made up of the previously identified V3 loop and the CD4-induced, chemokine receptor binding region.
The binding of gp120 to HP (Fig. 1, light lines) is known to involve the V3 loop of the glycoprotein. Previous studies indicated that this interaction did not follow a simple binding process, consistent with the view that gp120 could assume several distinct conformations, each of them having, in principle, the ability to bind to HP with distinct affinities. To investigate further the gp120/HP interaction, we made use of CD4, which constrains the glycoprotein into a specific “CD4-bound” conformation. For that purpose, gp120, either alone or complexed with sCD4, was injected over a HP-activated sensor chip. In the presence of sCD4, a dramatic increase in gp120 binding was observed (Fig. 1, heavy line). The injection of sCD4 alone did not produce any

**RESULTS AND DISCUSSION**

CD4 Enhances gp120 Binding to Heparin—The binding of gp120 to HP (Fig. 1, light lines) is known to involve the V3 loop of the glycoprotein. Previous studies indicated that this interaction did not follow a simple binding process, consistent with the view that gp120 could assume several distinct conformations, each of them having, in principle, the ability to bind to HP with distinct affinities. To investigate further the gp120/HP interaction, we made use of CD4, which constrains the glycoprotein into a specific “CD4-bound” conformation. For that purpose, gp120, either alone or complexed with sCD4, was injected over a HP-activated sensor chip. In the presence of sCD4, a dramatic increase in gp120 binding was observed (Fig. 1, heavy line). The injection of sCD4 alone did not produce any
significant binding response (not shown). The effect was maximal at low gp120 concentrations (1.25–5 nM; Fig. 1, A–C) at which virtually no binding could be detected in the absence of sCD4. HS has usually been considered as a preliminary binding site that would simply enhance HIV concentration at the cell surface before any other tight binding events take place (12). However, evidence in the present study showing that CD4 binding makes gp120 a much better HS ligand suggests rather that HS may interact with gp120 after the cell surface CD4 and, thus, that it may also play some role in post-attachment mechanisms (see below).

One of the conformational changes induced by CD4 is the shift of the V1/V2 and V3 loops. A first hypothesis would thus be that CD4-induced conformational changes of gp120 could favor HS binding by improving accessibility of the V3 loop and/or stabilizing its structure into a conformation better recognized by HS. However, molecular dynamic analyses indicated that if binding to CD4 substantially decreased the mobility of several regions of gp120, the V3 loop, although displaced, remains highly mobile (30). Moreover, a survey of the structural features of HS binding domains in a variety of proteins showed no absolute dependence on protein fold (31). Taken together, these data make it unlikely that V3 loop structural change would dramatically modify HS binding. Alternatively, sCD4 binding could trigger the display of new HS binding motifs within gp120.

The CD4i Region on gp120 Displays Features of a Typical Heparin Binding Site—A molecular modeling approach was used to locate putative HP binding sites on the core structure of gp120. This approach was initially developed and experimentally validated for analysis of the chemokine family, of which all members bind HP (24). Examination of the gp120 electrostatic surface, using the structural data of the core glycoprotein in the CD4-bound form, revealed that the CD4i region was the most likely HP binding site. In particular, amino acids Lys-121, Arg-419, Lys-421, and Lys-432 form a cluster of positively charged residues located between the stems of the V1/V2 and V3 loops and are organized as a possible HP binding site (Fig. 2A). This discontinuous surface has a linear shape extending up to 25 Å and can accommodate an oligosaccharide of eight sugar residues in length (Fig. 2, B and C). This observation was confirmed by a GRID analysis of the gp120 surface that was used to predict the most favorable anchoring position group, with an oxygen atom from a sulfate group serving as a probe for the calculation (not shown).

Heparin and Heparin-derived Oligosaccharides Inhibit the Binding of mAb 17b to gp120 in the Presence of sCD4—To assess the molecular modeling predictions of a HP-binding surface overlapping the CD4i region, we immobilized mAb 17b onto a sensor chip. mAb 17b belongs to the CD4i antibody family and recognizes an epitope on the gp120 bridging sheet that is exposed upon CD4 binding (32). Such mAbs, whose epitopes overlap the gp120 coreceptor binding domain, are widely used in interaction assays as coreceptor surrogates. In the absence of sCD4, a low concentration of gp120 (5 nM) failed to significantly interact with the 17b immobilized sensor chip surface (not shown). In contrast, the gp120/sCD4 complex, in which the CD4i region is optimally presented, bound well to 17b (Fig. 3A, top curve). Pre-incubation of gp120/sCD4 complexes with HP reduced binding to the 17b-activated surface, with an almost complete inhibition at concentrations as low as 16 nM (Fig. 3A, bottom curve). It is worth noting that among the four basic amino acids that we predicted would interact with HP, three of them (Lys-121, Arg-419, and Lys-421) are involved in mAb 17b recognition (7). Our experimental observation of mAb 17b/HP competition for binding to gp120 thus supports our model further. Similar results were obtained when the related mAb 48d was substituted for 17b and HS was used instead of HP (not shown).

Because HP is an extended molecule that can bridge several contact sites along the same protein, HP-derived oligosaccharides of defined length were used to compete with 17b for binding to the CD4i epitope. Fragments ranging from disaccharides (dp2) to octasaccharides (dp18) were prepared (19), pre-incubated with gp120/sCD4, and injected over the 17b-activated surface. Results indicated that the shortest fragments (dp2–6) did not efficiently compete with 17b for binding to gp120 and that octasaccharides (dp8) reduced the interaction to an intermediate degree, whereas longer oligosaccharides (dp10–18) efficiently inhibited the interaction (Fig. 3B). These results provide an experimental support for the model depicted in Fig. 2 in which an octasaccharide is the minimal length enabling interaction of the sugar carboxyl and sulfate groups with the basic amino acids within the CD4i region. Altogether, our results strongly suggest that the bridging sheet structure constitutes a new heparin binding domain on the protein. The structure of an unliganded simian immunodeficiency virus
(SIV) gp120 core has just been determined (33). Very interestingly, a comparison of the unliganded SIV gp120 core and the CD4-bound HIV gp120 core revealed that CD4 binding induces roughly half of the protein to refold. In particular, the bridging sheet structure found in the CD4-bound gp120, composed of four β-strands (β2, β3, β20, and β21), is absent in the unliganded state of the protein where β2-β3 and β20-β21 are spaced apart on the structure (33). Thus, the reorganization of gp120 induced by CD4 binding, rather than by exposing a previously masked surface, brings together the residues Lys-121 (in the β2-strand) and Lys-421 and Lys-432 (in the β20-β21 strands) that collectively form a new HP binding surface. Protein-HP binding is well known to induce protein conformational changes. The most documented example is that of antithrombin III, the binding of which to activated factor X is dependent on such HP-induced modifications (34). In contrast, the present work is probably one of the first examples in which a protein-induced conformational change primed another protein for HP recognition.

The importance of sulfate groups for CD4i domain binding has already been described, as the N-terminal domains of both CCR5 and CXCR4 that are involved in gp120 recognition feature a number of sulfotyrosines that contribute to HIV entry (35). Similarly, most of the anti CD4i antibodies display sulfotyrosines in their antigen binding sites (36). The binding of HS with the CD4i region may thus mimic, to some extent, the interaction taking place with CD4i antibodies or the N-terminal domain of the coreceptors.

Because the CD4i region comprises the coreceptor binding site, another important aspect of our study is that HP binds to the same surface as the CXCR4 coreceptor. This finding identifies the CD4i region as a potential target-using compound based on heparin-like structures (see below).

**The V3 Loop and the CD4i Epitope Form a Single Extended Heparin Binding Site on the gp120 Surface**—To study further the structural basis of gp120/HP interaction, the V3 loop that was missing in the available x-ray structure of gp120 was modeled and replaced on the protein core. Conformations of the V3 loop are available from NMR (23) and crystallography (37). In these structural studies the loop is bound to an antibody and not connected to the gp120 core, and, therefore, a molecular modeling approach was preferred here. Among the large panel of possible conformations, an extended β-hairpin close to the NMR conformation was selected. (Fig. 4). Such a shape was predicted to not interfere with oligomerization (18). Interestingly, in this model the basic sequence located at the V3 loop stem lined up with the HP binding site on the CD4i surface to form an extended binding domain. Various HP fragments selected from a library of oligosaccharide conformations were docked onto the gp120 V3 loop using the Tripos force field energy calculation. A tetradecasaccharide (dp14) enabled the bridging of CD4i and V3 loop binding sites (Fig. 4). Although V3 loop interaction with HP/HS is well established, the amino acids involved in the binding have not been identified. Our data suggest an involvement of Arg-304, Arg-306, Arg-308, and Arg-327 within the V3 loop. Because the V3 loop is predicted to be flexible, a variety of other shapes could be accommodated. Nevertheless, taking into account the flexibility of HP and the location of the CD4i region at the V3 loop stem, it is most likely that the polysaccharide could accommodate any V3 loop conformation and still interact with both binding regions. Experimental data will be required to confirm the importance of the amino acids for HP binding in both regions. These aspects will be investigated, either by using the “beads sequencing approach” reported recently (38) or by site-directed mutagenesis.

The physiological role of HS/CD4i interaction that we described here remains unclear but may shed light on the observation that cell surface HS enhances the infection of isolates that feature a highly positively charged V3 loop sequence (X4) but either has no effect on or inhibits infection by other (R5)
strains (39). It has been calculated that, over a distance and with a high charge, the contribution of the V3 loop dominates gp120 electrostatic potential, a feature that could be used for initial attraction of the viral Env toward the cell surface before CD4 engagement and is consistent with the HS-mediated increase in infectivity. However, at short distances and with a low V3 loop charge, the protein core contributes more significantly to the electrostatic potential (40). In such a situation, the only HS binding area, the CD4i epitope, would be exposed after CD4 engagement at the cell surface. The possible competition between coreceptor and HS for interaction with the CD4i surface may therefore explain the inhibitory activity of cell surface HS toward R5 HIV strains.

Finally, one implication of this study concerns polyanion-based inhibitors of HIV interactions with both attachment and entry receptors. Among the most promising recent approaches is the blockade of viral entry into target cells (41) and the polyanionic compounds that are about to enter phase III efficacy trials as microbicides (42). Based on the finding that the highly conserved coreceptor binding region directly interacts with HS after CD4 binding, we are currently investigating the HS toward R5 HIV strains.

REFERENCES

1. Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Herend, T., Gluckman, J. C., and Montagnier, L. (1984) Nature 312, 767–768
2. Ugolini, S., Mondor, I., and Sattentau, Q. J. (1999) Trends Microbiol. 7, 144–149
3. Berger, E. A., Murphy, P. M., and Farber, J. M. (1999) Annu. Rev. Immunol. 17, 657–700
4. Wyatt, R., and Sodroski, J. (1998) Science 280, 1884–1888
5. Sattentau, Q. J., Moore, J. P., Vignaux, F., Traincard, F., and Poignard, P. (1993) J. Virol. 67, 7383–7393
6. Wyatt, R., Moore, J., Accola, M., Desjardins, E., Robinson, J., and Sodroski, J. (1995) J. Virol. 69, 5723–5733
7. Rizzuto, C. D., Wyatt, R., Hernandez-Ramos, N., Sun, Y., Kwong, P. D., Hendrickson, W. A., and Sodroski, J. (1998) Science 280, 1949–1953
8. Basmaicogulari, S., Babcock, G. J., Van Ryk, D., Wojtowicz, W., and Sodroski, J. (2002) J. Virol. 76, 10791–10800
9. Roderiguez, G., Oravecz, T., Yanagishita, M., Bou-Habib, D., Mostowski, H., and Norcross, M. (1995) J. Virol. 69, 2233–2239
10. Bernfield, M., Gotte, M., Park, P. W., Reizes, O., Fitzgerald, M. L., Lincecum, J., and Zaks, M. (1999) Annu. Rev. Biochem. 68, 729–777
11. Spillmann, D. (2001) Biochimie (Paris) 83, 811–817
12. Oshiro, Y., Murakami, T., Matsuda, K., Nishioka, K., Yoshida, K., and Yamamoto, N. (1996) Microbiol. Immunol. 40, 827–835
13. McAdor, I., Ugolini, S., and Sattentau, Q. J. (1998) J. Virol. 72, 3623–3634
14. Saphire, A. C. S., Bobardt, M. D., Zhang, Z., David, G., and Gallay, P. A. (2001) J. Virol. 75, 9187–9200
15. Argyris, E. G., Acheampong, E., Nunnari, G., Mukhtar, M., Williams, K. J., and Pomerantz, R. J. (2003) J. Virol. 77, 12140–12151
16. Bobartid, M. D., Saphire, A. C., Hung, H. C., Yu, X., Van der Schueren, B., Zhang, Z., David, G., and Gallay, P. A. (2003) Immunity 18, 27–39
17. Moulard, M., Lortat-Jacob, H., Mondor, I., Roc, G., Wyatt, R., Sodroski, J., Zhao, L., Olson, W., Kwong, P. D., and Sattentau, Q. J. (2000) J. Virol. 74, 1948–1960
18. Kwang, P. D., Wyatt, R., Majeed, S., Robinson, J., Sweet, R. W., Sodroski, J., and Hendrickson, W. A. (2000) Structure Fold. Des. 8, 1329–1339
19. Sadir, R., Baleux, F., Grousidier, A., Imberty, A., and Lortat-Jacob, H. (2001) J. Biol. Chem. 276, 8288–8296
20. Tripos Inc. (1997) SYBYL Version 6.4, Tripos Inc., St Louis, MO
21. Walldherr-Teschner, M., Goette, T., Heiden, W., Knohlau, M., Vollhardt, H., and Brickmann, J. (1992) in Advances in Scientific Visualization, C. R., and Hin, A. J. S., eds) pp. 58–67, Springer, Heidelberg, Germany
22. Goodford, P. J. (1985) J. Med. Chem. 28, 849–857
23. Sharon, M., Kessler, N., Levy, R., Zolla-Pazner, S., Gorlach, M., and Anglister, J. (2003) Structure (Camb.) 11, 225–236
24. Lortat-Jacob, H., Grousidier, A., and Imberty, A. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 1229–1234
25. Vives, R. R., Sadir, R., Imberty, A., Bencuroso, A., and Lortat-Jacob, H. (2002) Biochemistry 41, 14779–14789
26. Gros, S., Petitou, M., Sizun, P., Perez, S., and Imberty, A. (1997) Biogorg. Med. Chem. 5, 1301–1309
27. Malovolov, D., Lindhardt, R. J., and Mayo, K. H. (1997) Biochem. J. 328, 51–61
28. Imberty, A., Bettler, E., Karababa, M., Mazeau, K., Petrova, P., and Perez, S. (1999) in Perspectives in Structural Biology (Vijayan, M., Yathindra, N., and Kolaskar, A. S., eds) pp. 392–409, Indian Academy of Sciences and Universities Press, Hyderabad, India
29. Clark, M., Cramer, R. D. I., and van den Opdenbosch, N. (1989) J. Comput. Chem. 8, 982–1012
30. Hsu, S. T., and Boosin, A. M. (2004) Proteins 58, 582–593
31. Mulloy, B., and Linhardt, R. J. (2001) Curr. Opin. Struct. Biol. 11, 623–628
32. Wyatt, R., Kwong, P. D., Desjardins, E., Sweet, R. W., Robinson, J., Hendrickson, W. A., and Sodroski, J. G. (1988) Nature 335, 705–711
33. Chen, C., Vogan, E. M., Gong, H., Skehel, J. J., Wiley, D. C., and Harrison, S. C. (2005) Nature 433, 834–841
34. Olson, S. T., Bjork, I., Sheffer, R., Craig, P. A., Shore, J. D., and Choy, J. (1992) J. Biol. Chem. 267, 12528–12538
35. Farzan, M., Mirzabekov, T., Kolchinsky, P., Wyatt, R., Cayababv, M., Gerard, N. P., Gerard, C., Sodroski, J., and Choe, H. (1999) Cell 96, 667–676
36. Choe, H., Li, W., Wright, P. L., Vasiliou, N., Venturi, M., Huang, C. C., Grunudner, C., Dorfman, T., Zwick, M. B., Wang, L., Rosenberg, E. S., Kwong, P. D., Burton, D. R., Robinson, J. E., Sodroski, J. G., and Farzan, M. (2003) Cell 114, 161–170
37. Stanfield, R. L., and Bogin, D., Williams, C., Zolla-Pazner, S., and Wilson, I. A. (2004) Structure (Camb.) 12, 193–204
38. Vives, R. R., Cruhlet, E., Andrieu, J.-P., Gagnon, J., Rousselle, P., and Lortat-Jacob, H. (2004) J. Biol. Chem. 279, 54327–54333
39. Zhang, Y.-j, Hatzizianou, T., Zang, T., Braaten, D., Luban, J., Goff, S. P., and Bieniasz, P. D. (2002) J. Virol. 76, 6332–6343
40. Kwang, P. D., Wyatt, R., Sattentau, Q. J., Sodroski, J., and Hendrickson, W. A. (2000) J. Virol. 74, 1961–1972
41. Moore, J. P., and Stevenson, M. (2000) Nat. Rev. Mol. Cell Biol. 1, 40–49
42. Stone, A. (2002) Nat. Rev. Drug Discov. 1, 977–985