

THE ROLE OF CELL SURFACE HEPARAN SULFATE PROTEOGLYCAN (HSPGs) IN HIV-1 VIRAL ASSEMBLY AND HIV-1 TAT CELLULAR UPTAKE

Elias G. Argyris¹, Marie E. Meyer², Kevin Jon Williams² and Roger J. Pomerantz¹

The Dorrance H. Hamilton Laboratories, ¹ Center for Human Virology, Division of Infectious Diseases;

² Division of Endocrinology, Diabetes and Metabolic Diseases, Department of Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA USA



ABSTRACT

Recent studies have demonstrated the significance of cell surface HSPGs in HIV-1 infection, as well as the cellular uptake of Tat, the transactivator protein of HIV-1. In this study we examined: a) The hypothesis that HIV-1 in the process of viral budding incorporates HSPG molecules, and b) the role of perlecan, the only HSPG expressed on the cell surface of the colon carcinoma cell line WiDr, in HIV-1 Tat uptake and internalization. Our studies show that: a) Purified HIV-1 particles that were generated from ³⁵SO₄-labeled CEM cells are highly sulfated, and further analysis by DEAE-chromatography and SDS-PAGE provides strong support that sulfation of HIV-1 is due to incorporation of ³⁵S-HSPG molecules, which may take place during viral budding. b) WiDr cells are able to bind and internalize substantial amounts of extracellular ¹²⁵I-Tat, consistent with a role for the perlecan HSPG. A minority of the internalized ligand undergoes degradation, while most of ¹²⁵I-Tat remains in the cells even after 22 hours. Our results strongly suggest the incorporation of HSPGs into the viral envelope during viral budding. Further characterization of these molecules will provide valuable information on viral structure and pathogenicity. In addition, our results provide strong support for the participation of perlecan, the only HSPG expressed on the surface of the colon carcinoma cell line WiDr, in HIV-1 Tat uptake and internalization.

MATERIALS & METHODS

Materials.

Recombinant HIV-1 Tat was obtained from Fitzgerald Industries International, Inc. (Concord, MA), WiDr human colon carcinoma cell line (also known as HT-29, that synthesizes perlecan but no other proteoglycans) from ATCC (Manassas, VA), Na₂³⁵SO₄ and Na ¹²⁵I from ICN Pharmaceuticals, Inc. (Costa Mesa, CA), and heparitinase (heparinase III) from Sigma (St. Louis, MO).

HIV-1 Infection Assays/Cell ³⁵S-labeling.

a). CEM cells (1-2x10⁶) were infected with 1 ng p24 antigen of the T-tropic HIV-1 strain NL4-3. Cells were then metabolically labeled with 40 μCi of Na₂³⁵SO₄ and transferred to ELISA plates, in RPMI-1640 medium supplemented with 10% FBS at 37°C. Seven days post-infection virus-containing supernatant was collected, purified and further analyzed.

Purification of HIV-1 particles and proteoglycan/glycosaminoglycan molecules.

HIV-1 was purified by column chromatography using CL-2B Sepharose. Eluted fractions were ³⁵S scintillation counted, and analyzed for HIV-1 p24 antigen (NEN HIV-1 p24 Antigen ELISA detection system). ³⁵S-HSPG molecules incorporated into HIV-1 particles were further purified by DEAE chromatography (DEAE Bio-Gel A Agarose, Bio-Rad) and analyzed by SDS gel followed by autoradiography.

HIV-1 Tat Iodination and Purification.

Recombinant HIV-1 Tat (25 μg) was ¹²⁵I-labeled using iodo-beads iodination reagent (Pierce, Rockford, IL) and ¹²⁵I-Tat was further purified through Sephadex G-25 column (PD-10 Desalting columns, Amersham Pharmacia Biotech, Piscataway, NJ) and dialysis against PBS buffer.

Cellular Uptake and Degradation of ¹²⁵I-Tat.

WiDr cells were incubated in serum-free medium (DMEM) with ¹²⁵I-Tat at 37°C for 5 and/or 22 hours. HIV-1 Tat cellular uptake and internalization was analyzed by quantitation of surface-bound (heparin-releasable), intracellular (heparin-resistant), and degraded (assessed by trichloroacetic acid-soluble, CHCl₃-insoluble radioactivity in media) ¹²⁵I-Tat. To verify the role of heparan sulfate side chains of perlecan in binding and catabolism of ¹²⁵I-Tat, WiDr cells were pretreated for 1 hour at 37°C with heparitinase (5.5 units/ml), and heparitinase was kept in the incubation medium with labeled ligand until the end of the experiment, to prevent reassembly of HS side chains.

RESULTS

³⁵S-labeled HIV-1 particles, generated from metabolically labeled CEM cells, and purified by column chromatography using CL-2B Sepharose, are shown in **Figure 1**. Analysis of collected gel-filtration ³⁵S-labeled fractions for HIV-1 p24 antigen revealed the presence of ³⁵S-labeled HIV-1 particles in fractions 9-13 (**Figure 2**), thus strongly suggesting the incorporation of HSPGs into the viral envelope during viral budding. Further analysis by DEAE-chromatography (**Figure 3**) and SDS-PAGE (data not shown) was consistent with the presence of ³⁵S-HSPG molecules on the viral particles.

FIGURE 1:

Analysis of purified by Gel Filtration ³⁵S-fractions HIV-1

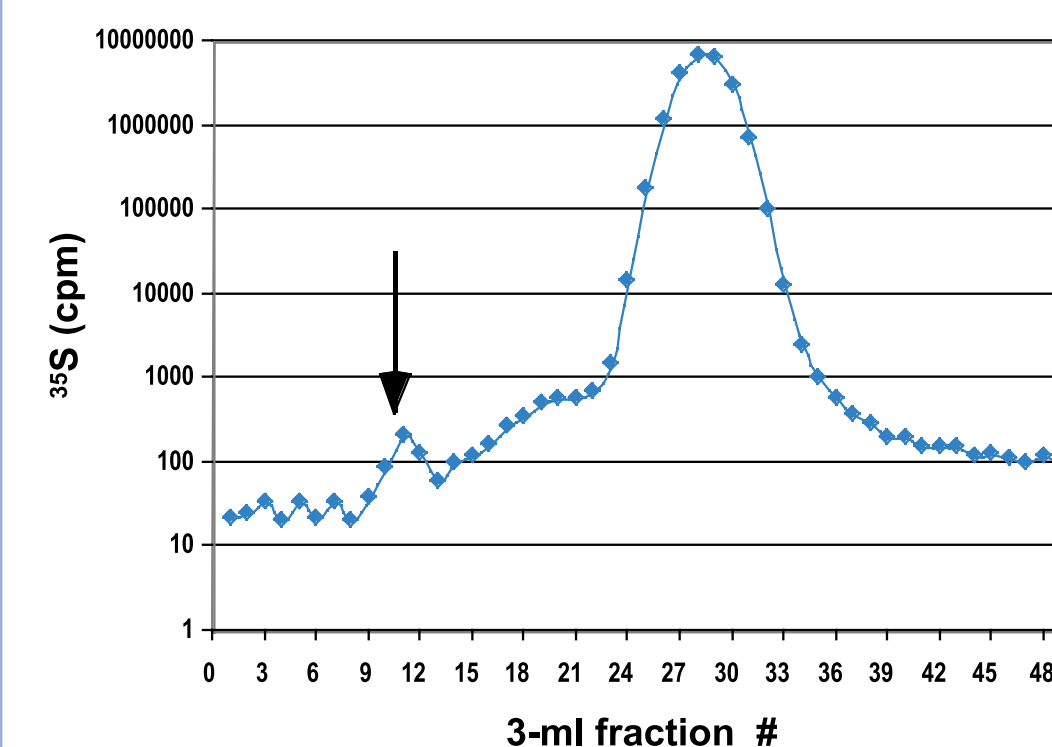


FIGURE 2:

Correlation between Gel-filtration ³⁵S-fractions and HIV-1 p24 Antigen

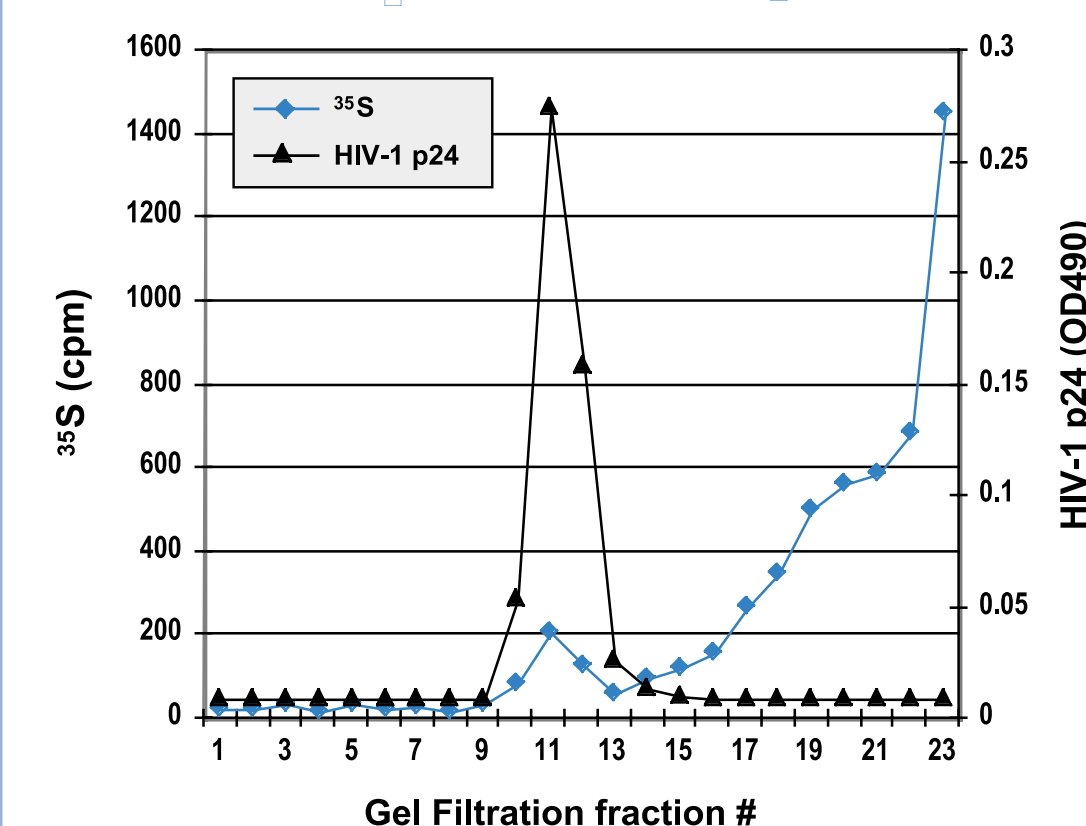


FIGURE 3:

DEAE-purification of ³⁵S-HSPGs incorporated into HIV-1

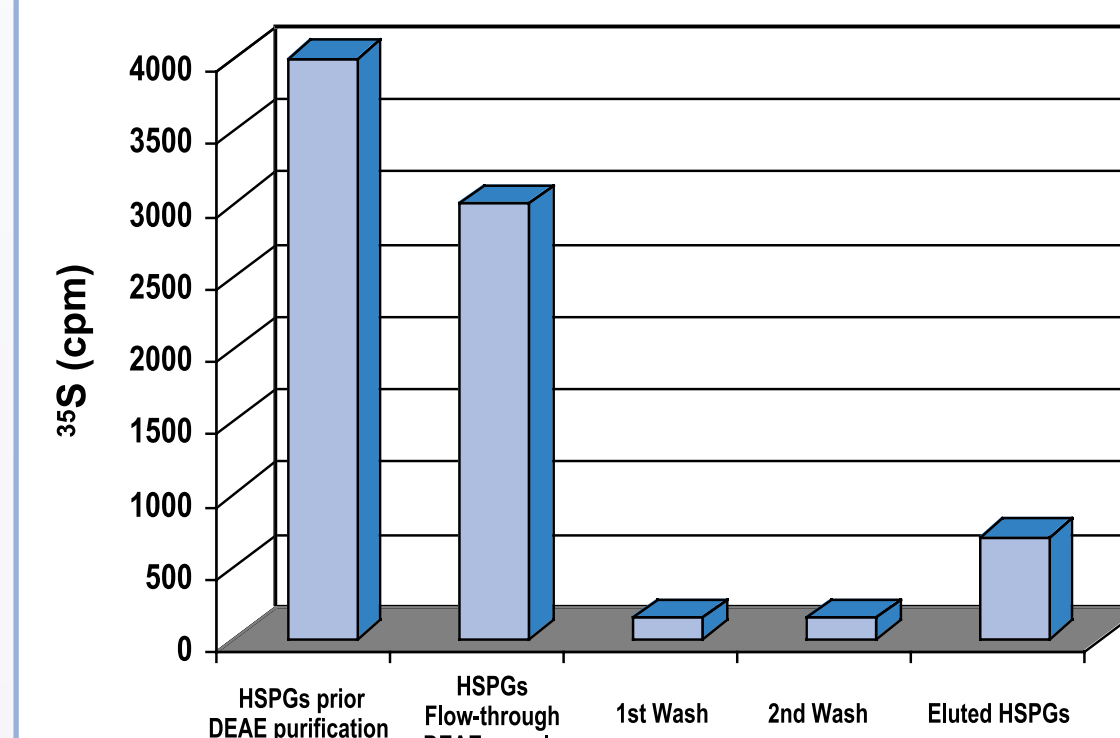


FIGURE 4:

Participation of Perlecan in HIV-1 Tat catabolism (5 Hours)

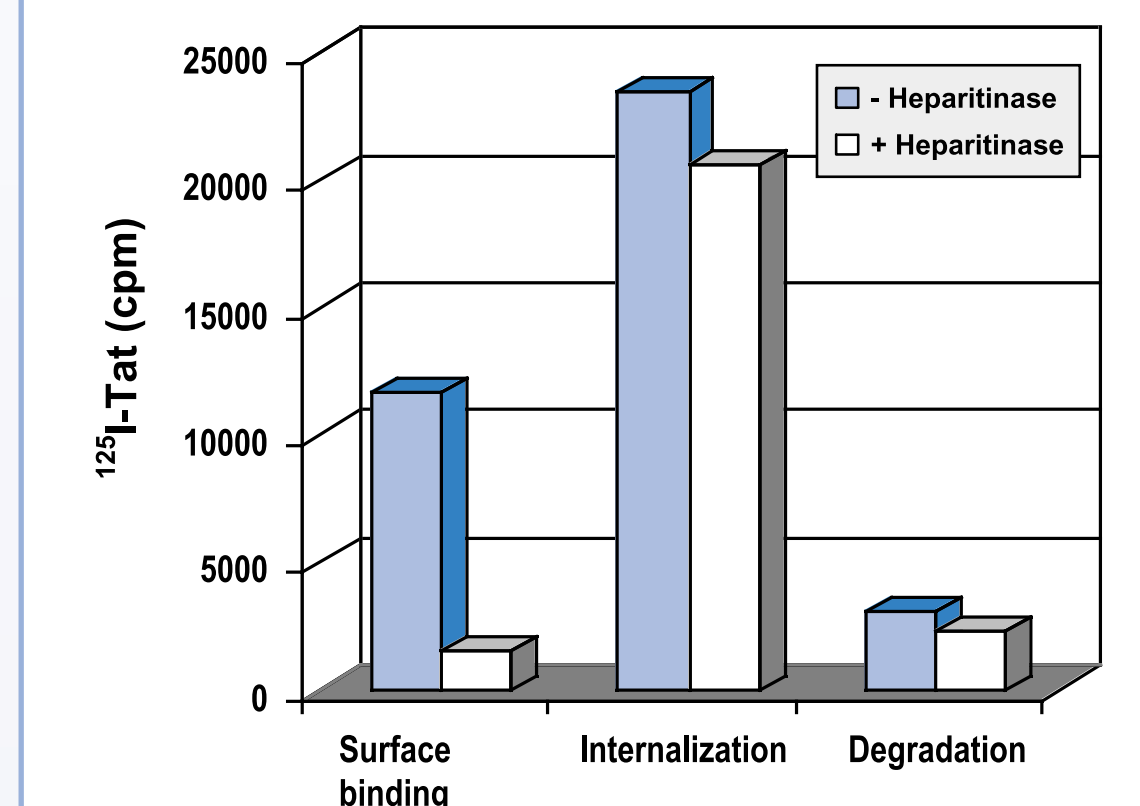
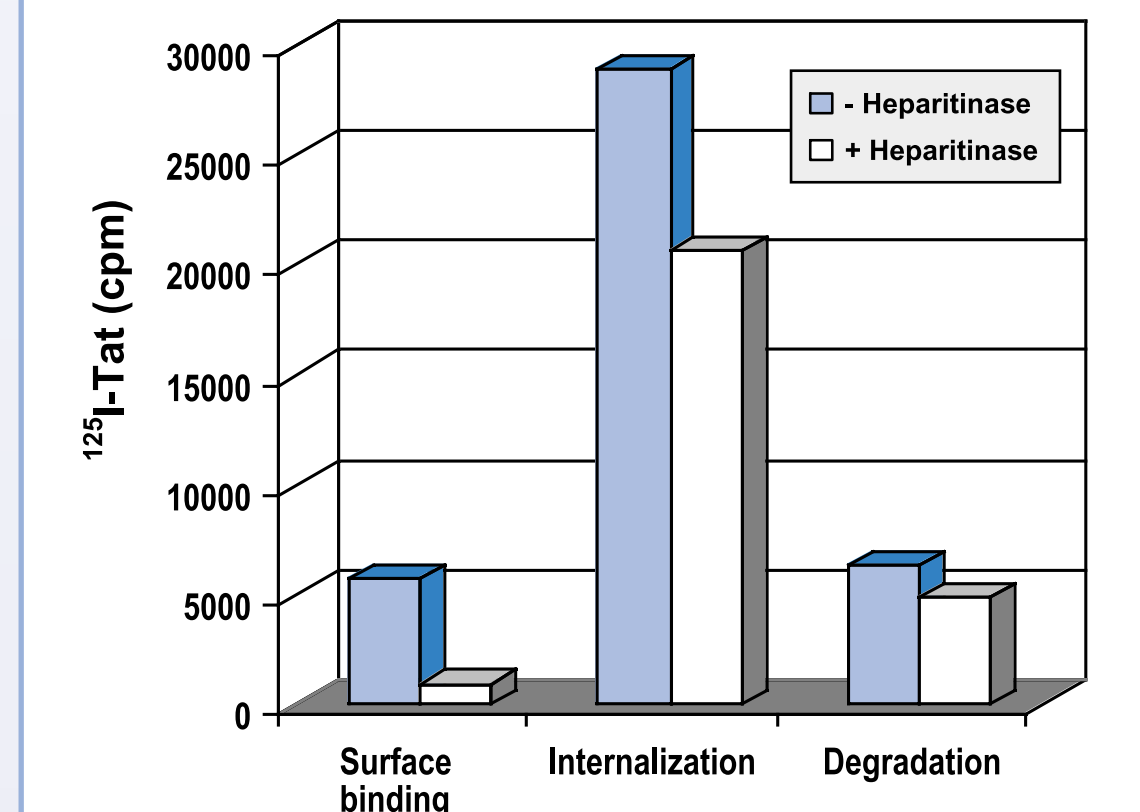


FIGURE 5:

Participation of Perlecan in HIV-1 Tat catabolism (22 Hours)



INTRODUCTION

Recent studies have demonstrated the significance of cell-surface HSPGs and lipid rafts in HIV-1 infection. It was shown that cell-surface heparan sulfate molecules facilitate HIV-1 entry into certain cell lines but not primary lymphocytes, and most importantly a single family of HSPGs, the syndecans, efficiently mediate HIV-1 attachment and represent an abundant class of attachment receptors on macrophages. In addition, there is strong evidence that cholesterol-enriched microdomains ("lipid-rafts") at the plasma membrane play a critical role in HIV-1 assembly and release. Furthermore recent studies demonstrate that cell-surface HSPGs are important for the cellular uptake and internalization of Tat, the transactivator protein of HIV-1. In this study we examined a) whether HIV-1 in the process of viral budding incorporates integral membrane HSPGs that are known to localize to rafts, and b) the role of perlecan, the only HSPG expressed on the cell surface of the colon carcinoma cell line WiDr, in HIV-1 Tat uptake and internalization.

CONCLUSIONS

- The presence of ³⁵S-molecules on HIV-1 particles strongly suggests the incorporation of HSPGs into the envelope during viral budding. Further characterization of these molecules will provide valuable information on viral structure and pathogenicity.
- Our results provide strong support for the participation of perlecan, the only HSPG expressed on the surface of the colon carcinoma cell line WiDr, in HIV-1 Tat uptake and internalization. Further experimentation is required to fully characterize the role of HSPG in extracellular HIV-1 Tat uptake.