



Reactivity and utility of pseudoproteoglycan probes that simulate proteoglycan supramolecular structure

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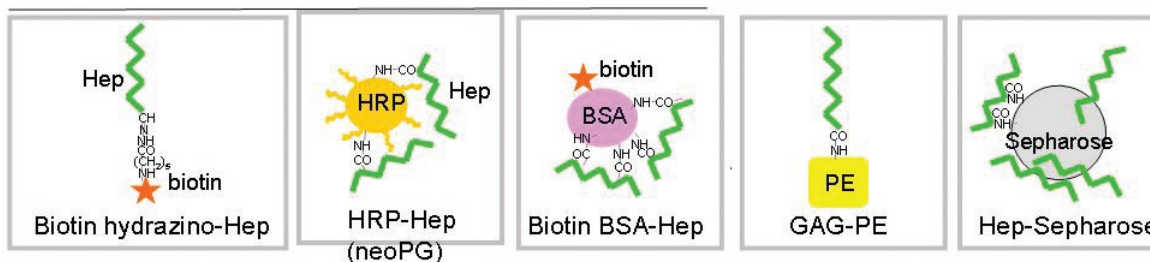
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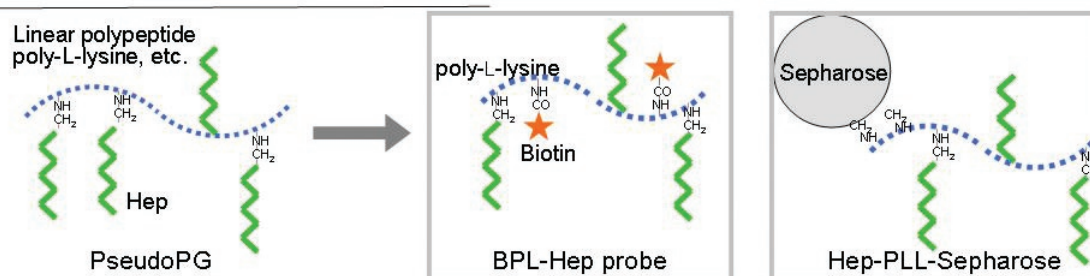
Haruko Ogawa graduated from Department of Chemistry, Ochanomizu University and studied at the Graduate School of Sciences at the same university. She received her Ph.D. in 1986 from the University of Tokyo, and continued her research as an assistant professor, and since 1992, as an associate professor at Ochanomizu University. During this period, she contributed in particular to the development of adsorbents for purification of carbohydrate-binding proteins and the biochemical

characterization of novel lectins. In 1990, she worked as a visiting scholar in the Department of Biochemistry and Biophysics of the University of California at Davis (Prof. M.E. Etzler). In 1998, she moved to her current affiliation at Ochanomizu University, and she became a full professor in 2005. Research projects within her group are focused on the glycan functions of glycoconjugates, especially the modulatory roles of glycans in the extracellular matrix glycoproteins during liver regeneration, the elucidation of novel carbohydrate-recognition systems, including the carbohydrate-binding activities of pancreatic enzymes, and on developing various glycoprobes for studying the glycobiology.

Conventionally prepared probes and adsorbent



PseudoPG probe and adsorbent



Scheme 1 Probes and adsorbents of conventional methods and pseudoPG

A proteoglycan (PG) is a supermolecule consisting of glycosaminoglycan (GAG) chains attached to a core protein, which binds link proteins and hyaluronans. PGs have signaling roles and cell modulatory functions in the extracellular matrix and at the cell surface. Conventionally, PG-binding proteins are studied using probes of GAG chains labeled with a functional tag such as a fluorescent chromogen, biotin, or epitope for immunoenzymatic detection, or conjugated with a hydrophobic group to immobilize them on the solid phase (Fig. 1). Some of these probes may possess reactivities with biological ligands other than those of PGs because the probes are dissimilar from the higher-order structure of natural PGs. In this study, to elucidate the function of the higher-order structures of PGs, pseudoPGs that simulate the structure of a PG monomer with GAG side chains attached to a linear polypeptide strand were prepared for use as probes and affinity adsorbents to search for and locate PG-binding substances. The affinities of the pseudoPG probes for several proteins were compared with those of other probes, and the pseudoPG probes were used to screen and separate ligand proteins in rat brain extracts.

Preparation of probes and adsorbents. GAGs were coupled with poly-L-lysine (PLL) or linear polyacrylamide by reductive amination (PLL-GAG or PAA-GAG, respectively) and biotinylated; then, the remaining amino groups were blocked by *N*-acetylation to obtain biotinyl poly-L-lysine (BPL)-GAG or biotinyl polyacrylamide (BPA)-GAG probes. The coupling of heparin to PLL and formation of BPL-Hep was detected by SDS-PAGE and size-exclusion chromatography-multi-angle laser-light scattering (SEC-MALLS). As a control, a BPL probe without GAGs was prepared. A biotin hydrazino-heparin (Bio-Hep) probe consisting of a simple Hep chain coupled with biotin hydrazide,

and a neoproteoglycan (neoPG) probe, which is Hep coupled to horseradish peroxidase (HRP-Hep) with the aid of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), were prepared for comparison. Hep-PLL was immobilized to formyl-Sepharose by reductive amination to obtain Hep-PLL-Sepharose (bound Hep 1 mg/g gel). For comparison, Hep-Sepharose was prepared by directly immobilizing Hep chains to amino-Sepharose using EEDQ (bound Hep 7 mg/g gel). As a control, PLL-Sepharose without GAGs was prepared.

Interaction studies by ELISA and SPR. Binding studies by solid phase assay indicated that the reactivity of the heparin-binding proteins changes between BPL-Hep and Bio-Hep. SPR showed that the pseudoPG, BPL-Hep probe exhibited significantly enhanced affinity to several protein ligands compared to Bio-Hep.

Detection of Hep-binding proteins. Western blotting showed that HRP-Hep and BPL-Hep probes bound to more protein bands of rat brain extracts than the biotin hydrazino-Hep probe did. Remarkably, several proteins in rat brain extracts bound specifically to either the BPL-Hep or the Bio-Hep probe, indicating that the Hep-binding proteins exhibit recognition for a higher-order structure of PG.

Separation by affinity chromatography. Extracts from normal rat brain were applied to affinity chromatography on columns of Hep-PLL-Sepharose or Hep-Sepharose, and the proteins that bound to either of the two adsorbents were identified by direct sequencing.

This study indicates the biological significance of the proteoglycan structure in carbohydrate recognition and the utility of pseudoPG probes for the detection and separation of proteins and recognition of events that involve higher-order PG structures.

Keywords : Pseudoproteoglycan, glycoprobe, affinity adsorbent, heparin-binding, higher-order structure