SEC MALS Characterization of Heparin-BSA

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Fina Biosolutions provides conjugates of heparin, including biotin-heparin, fluorescent heparin and BSA-heparin. Our conjugates are synthesized by modifying only the polymer end, leaving the heparin polymer chain intact. This note describes the controlled synthesis of FinaBio's heparin-BSA and conjugate characterization by the SEC-MALS analysis.

About Heparin/Introduction. Heparin[1] is a highly sulfated glycosaminoglycan (Figure 1) molecule which is mainly used as an injectable anticoagulant but recent reports indicate that the molecule also possesses anti-inflammatory activity. Heparin has the highest negative charge density of any known biological molecule. Native heparin is a heterogeneous mixture of polymers with varying sulfate group patterns, and a molecular weight range from 3 to 30 kDa, although the average molecular weight of most commercial heparin is in the range of 12 to 15 kDa. Heparin is typically obtained from porcine or bovine mucosal tissue.

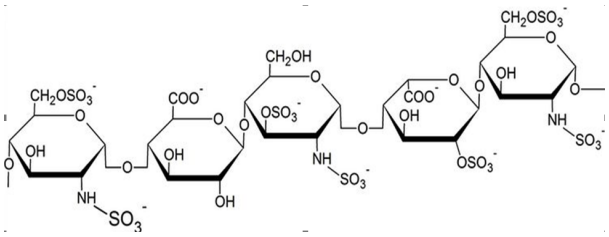

FIGURE 1. TYPICAL HEPARIN STRUCTURE

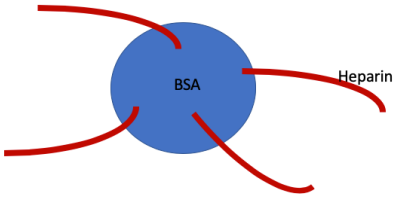

FIGURE 2. END-LINKED HEPARIN

Heparin Modification. Fina Biosolutions heparin conjugation involves functional group transformation of the terminal reducing end of the polymer. The modified end is then linked to a protein and/or other probing agent. This method has been shown to be superior to intrachain periodate oxidation derivatization and conjugation approach, which involves more extensive modification of the heparin backbone and leads to a lattice conjugate. In contrast, end-linking of the heparin to the protein leads to a "star" configuration (Figure 2). End-linked heparin derivatives were found to have both higher affinity and higher binding capacity for many heparin binding proteins than lattice conjugates[2].

Conjugate optimization. In order to study the extent of heparin labelling using conjugation via the end terminal, end-activated heparin was reacted with BSA using 0, 5, 10 and 20-fold molar excess of heparin to BSA. The conjugates were then analyzed to determine the average molecular weight and the heparin:protein ratio.

Molecular weight determination. We used analytical size exclusion chromatography coupled with multi-angle light scattering (SEC MALS) to determine the absolute molecular weight of heparin and heparin-BSA, as well as the heparin:BSA ratio. As size exclusion chromatography

separated unconjugated heparin and BSA from conjugate, the analysis could be done prior to purification. SEC MALS is a technique for determining the absolute molecular weight of biomolecules[3]. The MW is derived using the UV absorbance, refractive index and light scattering detectors, without the need for a standard. The only experimentally derived value needed is the incremental refractive index, (dn/dc), and the extinction coefficient of both the protein and the modifier. The analytical results are shown in Table 1 and plotted in Figure 3.

	MW	Mole Hep:BSA	mg Hep/mg BSA
Heparin	16.5 kDa	n/a	n/a
Hep-BSA 5x	187.3 kDa	3.2	0.82
Hep-BSA 10x	262.8 kDa	6.1	1.56
Hep-BSA 20x	338.9 kDa	9.2	2.4

Table 1. SEC MALS analysis of heparin-BSA conjugates. End-derivatized heparin was conjugated to BSA at a starting molar ratio of 5, 10 or 20:1.

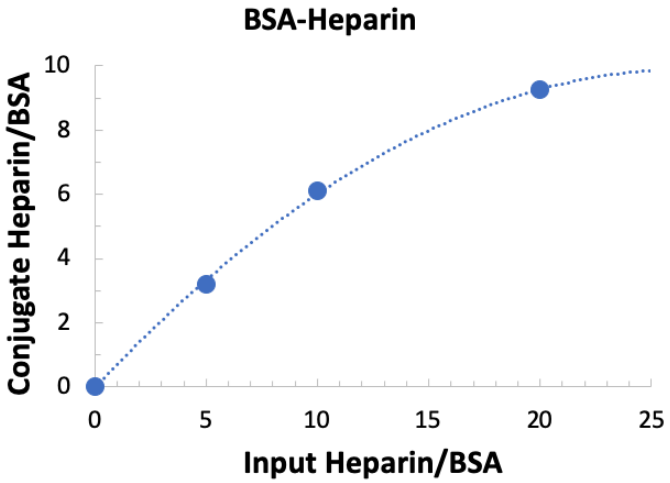

Figure 3. Moles heparin per mole BSA in conjugate vs starting ratio as determined by SEC MALS

Size exclusion chromatography (SEC) separates molecules according to their hydrodynamic volume while multi-angle light scattering is used to determine the absolute molecular weight of the eluting volume. Figure 4 illustrates the molecular weight of the eluting peaks for each of the 3 conjugates as well as the starting heparin and BSA. The relatively flat lines for the molecular weights indicate reasonably good homogeneity for the conjugates.

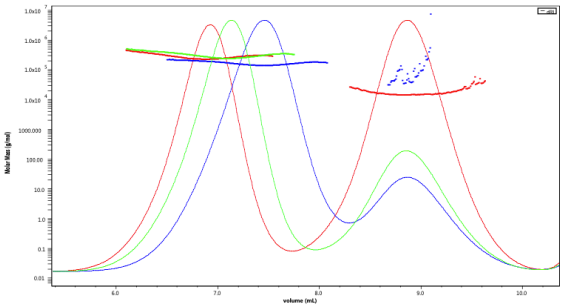

Figure 4. Molar volume of BSA-Heparin conjugates along with that of BSA and Heparin-BSA conjugates, as determined by SEC HPLC MALS.

The x-axis of Figure 4 is the elution volume, with species with a larger hydrodynamic radius eluting earlier. The peaks represent the refractive index signal. The y-axis scale is the molecular weight across the eluting peak. Peaks to the left are conjugates and the peak on the right is BSA.

Conclusion: Fina Biosolutions approach to mono functionalize heparin at the reducing end of the molecule followed by conjugation to a protein and/or a probing agent provides a well-defined and controlled conjugate. In this case study we showed that end-linked heparin-BSA conjugates could be prepared and easily characterized by SEC-MALS (Figure 4) to determine the average molecular weights and the heparin: BSA ratio.

Related Products: Heparin-BSA, monobiotin-Heparin, Alexa488-Heparin

References

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2. Osmond RIW, K. W., Spencer ES, and Coombe DR, Protein–heparin interactions measured by BIAcore 2000 are affected by the method of heparin immobilization. *Analytical biochemistry* **2002,** *310*, 199.
3. Kendrick, B. S.; Kerwin, B. A.; Chang, B. S.; Philo, J. S., Online size-exclusion high-performance liquid chromatography light scattering and differential refractometry methods to determine degree of polymer conjugation to proteins and protein-protein or protein-ligand association states. *Analytical biochemistry* **2001,** *299* (2), 136-46.

About Fina Biosolutions Services: Fina Biosolutions is a leader in providing high-quality base and functionalized dextran products. Since our founding, we have implemented rigorous quality controls to ensure that our dextran polymers and conjugates meet strict requirements for molecular weight, polydispersity, the degree of functionality and reproducibility of manufacturing. With input from our customers, we have also recognized the need in offering our expertise in polymer sciences to the scientific community at large. We offer services such as polymer characterization, custom functionalization, conjugation and end-product characterization.