Molecular Weight Distribution of *Nephila Clavipes* Dragline Silk

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Introduction. Among the several classes of structural proteins produced in nature, silklike proteins have been of special interest to the materials community because of their unusual and in some cases unique mechanical properties. More recently, much attention has been focused on the filaments produced by orb weaving spiders, selected examples of which have combinations of strength and toughness unmatched in man-made fibers. With the advent of powerful methodologies to accurately sequence and characterize protein polymers, a more thorough understanding of structure/property relationships in these important evolutionary materials is now possible.

The molecular weight of *Nephila clavipes* dragline silk has been determined by gel electrophoresis and reported as 275 000[1] and 320 000 Da.[2] In this study the molecular weight was measured using size-exclusion chromatography with hexafluoroisopropanol containing 10 mM sodium trifluoroacetate as the mobile phase. This has been found to be a good solvent system for the analysis of synthetic polyamides[3] and is also a good solvent for silk proteins. An instrument combining size-exclusion chromatography with viscosity and light scattering detectors developed for polyamide analysis was used. The instrument measures directly the absolute molecular weight distribution without the need for molecular weight calibration standards, and only a relatively small sample amount is required (~100 μg).

Experimental Section. Preparation of Dragline Silk Samples. Silk protein was removed from freshly excised glands of two female *N. clavipes* and dissolved in hexafluoroisopropanol (HFIP) at concentrations of approximately 1 mg/mL. For the dragline fiber samples, live female spiders (*N. clavipes*) with emergent dragline filaments were anesthetized with CO_2. The specimens were restrained on a glass plate and dragline filaments mechanically drawn from the major ampullate gland using a small motorized windup. The filaments were used without further preparation for these studies. Three samples of dragline silk were characterized: one was fresh (same day), and the other two were obtained over the course of several weeks. Each sample was analyzed within 2 days of being placed in HFIP.

Size-Exclusion Chromatography. The size-exclusion chromatograph consisted of a Waters Model 590 pump (Waters Associates, Milford, MA) and three Shodex linear size-exclusion columns in series, containing cross-linked styrene–divinylbenzene 5 μm packing, packed in hexafluoroisopropanol (Showa Denko Co., obtained from Waters Associates). Each column was 300 × 8.0 mm i.d, a guard column was also used (50 × 8.0 mm). The mobile phase was hexafluoroisopropanol (99.5+%, Aldrich Chemical Co.), containing 0.010 M sodium trifluoroacetate. The flow rate was 1.0 mL/min. The concentration detector was a Waters Model 410 differential refractive index detector. The viscometer was a Model 110 (Viscotek Corp., Houston, TX), and the laser light scattering photometer was a Model F with a 633-nm, 5-mW helium–neon laser (Wyatt Technology Corp., Santa Barbara, CA). All measurements were made at 30 °C. A Hewlett Packard Model 1050 autosampler was used to inject 100–200 μL of the solutions onto the chromatograph. The data were collected and processed using in-house software. The specific refractive index increment (dn/dc) of silkworm silk was measured previously using a Chromatix KMX-16 (LDC Milton Roy) as 0.236 mL/g at 633 nm. For the spider silk the value of dn/dc was estimated at 0.235 mL/g from the calibrated differential refractometer response to an injection of known mass for the dragline fibers. The second virial coefficient was determined for the major ampullate gland silk from the peak light scattering intensity for a series of different injected masses and was found to be 3.0×10^{-3} mL·mol/g^2.

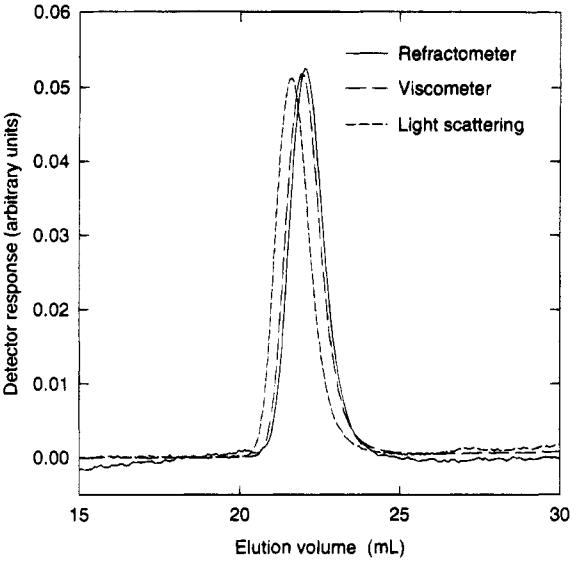

Figure 1. Chromatograms for freshly spun dragline silk protein. From left to right the tracings correspond to the responses from the light scattering, viscosity, and differential refractive index detectors. Peaks are normalized to the same area.

Results and Discussion. The tracings from the light scattering, viscosity, and refractive index detectors for the silk extracted from the major ampullate gland are shown in Figure 1. The detectors show single narrow peaks, indicating a narrow molecular weight distribution. For all samples, a linear decrease in log molecular weight with increasing elution volume was measured, indicating that the separation was based on size exclusion and that there were no interactions with the column packing. The solutions appeared stable and repeat injections of the silk solutions over a period of 2 weeks gave the same molecular weight distributions. The moments of the molecular weight distributions, molecular weight polydispersities, and intrinsic viscosities are given in Table 1. The differential molecular weight distributions for the five samples are shown in Figure 2. Typical relative standard deviations are 1–2% for M_w, M_z, and $[\eta]$ and 5–10% for M_n. For the samples from the glands the radius of gyration was measured from the scattering asymmetry and was found to be 57 nm for the major ampullate silk and 31 nm for the minor ampullate silk.

The silk taken directly from the glands has molecular weights much higher than reported previously and has

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Table 1. Molecular Weight Distribution and Intrinsic Viscosity Results

sample	M_n	M_w	M_z	M_w/M_n	M_z/M_w	$[\eta]$, dL/g
major ampullate gland silk	720 000	740 000	760 000	1.03	1.02	9.85
minor ampullate gland silk	270 000	290 000	300 000	1.04	1.04	4.34
freshly spun dragline silk	520 000	580 000	620 000	1.11	1.07	8.26
dragline silk sample 1	340 000	470 000	600 000	1.38	1.27	6.39
dragline silk sample 2	96 000	240 000	380 000	2.45	1.62	4.03

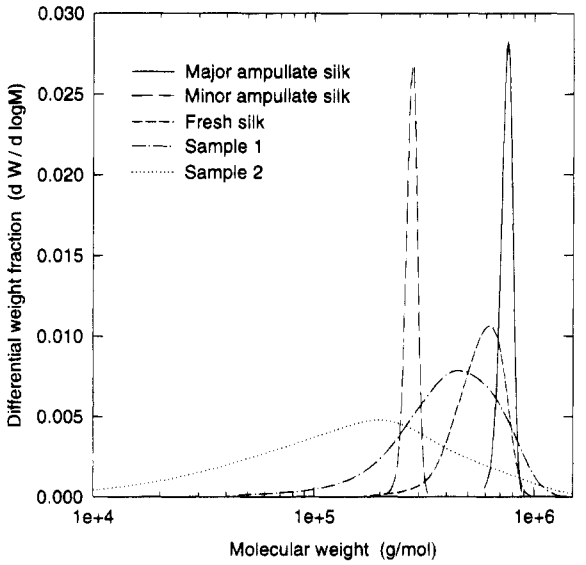

Figure 2. Differential molecular weight distributions for the five samples. The two narrow peaks correspond to the minor and major ampullate gland silk proteins, and the three broader peaks are for the fibers.

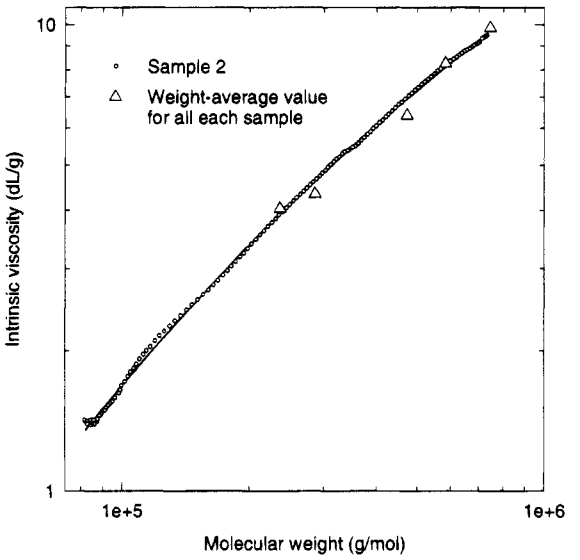

Figure 3. Intrinsic viscosity–molecular weight plots for the average values of the five samples and for data for each elution slice of sample 2.

very narrow molecular weight distributions. The freshly spun dragline silk is 30% lower in molecular weight than the silk from the major ampullate gland and has a significantly broader molecular weight distribution. The oldest dragline silk sample has a molecular weight that is 13% of the major ampullate gland silk and a polydispersity greater than a most probable distribution. The highest limit of the molecular weight distributions of the three dragline samples coincides with the high limit of the gland silk (Figure 2). These results indicate that there is degradation of the molecules both during the spinning process and also subsequent to spinning. The polydispersity value greater than 1 observed for spider silk is in accord with previous studies, suggesting that the natural fiber is comprised of more than a single protein. Cloning and sequencing of dragline silk DNA in *N. clavipes* have revealed the presence of at least two proteins.[4] Stubbs *et al.*[5] have extracted selected silk fibers from other cylindrical fiber-producing glands and have shown such fibers to have well-defined composite structures with as many as 10 major protein components. *Bombyx mori* fibroin has also been shown to consist of subunits bound by noncovalent bonds.[6-8] If the composite structures are partially broken up during spinning, the resolution of size-exclusion chromatography may not be sufficient to resolve each structure and an apparently continuous molecular weight distribution would be detected. The polydispersity in the silk does not appear to come from the mixture of minor and major ampullate silk as these are both so narrow and different in molecular weight distribution that any mixture would have a clear bimodal distribution. As reported previously,[9] proteins rich in glycine and alanine can migrate anomalously during gel electrophoresis, often resulting in an overestimate of the actual protein molecular weight. However, in general, the values found here are larger than previously reported, supporting the conclusion that the silk is composed of multiple protein subunits.

The Mark–Houwink plot of intrinsic viscosity against weight-average molecular weight for all five samples is shown in Figure 3. In addition, the intrinsic viscosity and molecular weight data for each elution slice measured for dragline fiber sample 1 are shown. All the data fall on the same straight line described by

$$[\eta] = 1.8 \times 10^{-4} M^{0.81}$$

The exponent is on the high side of the range for flexible chains in a good solvent and may indicate some chain stiffness even at these high molecular weights.

Conclusions. In conclusion, size-exclusion chromatography in hexafluoroisopropanol with 0.01 M sodium trifluoroacetate has been used to determine the molecular weight distribution of *N. clavipes* dragline silk. The proteins appear to form a single, high molecular weight complex in the glands. After the dragline fiber is spun, the molecular weight has decreased and there is some broadening of the distribution. Older fiber samples appear to be more polydisperse and have lower molecular weights.

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