Supplementary Materials

Comparison of Electrophoretic and Hydrodynamic Injection

Figure S1 compares some electropherograms that were obtained in this study when using electrophoretic injection versus hydrodynamic injection. The electrophoretic injection results were obtained using a 4.0 mg/L standard solution of normal and purified AGP that was applied at an injection voltage of -5 kV for 5 min. The results obtained by hydrodynamic injection represent an injection made at 0.5 psi for 3 s for an aqueous standard containing 4.0 g/L of normal and purified AGP.

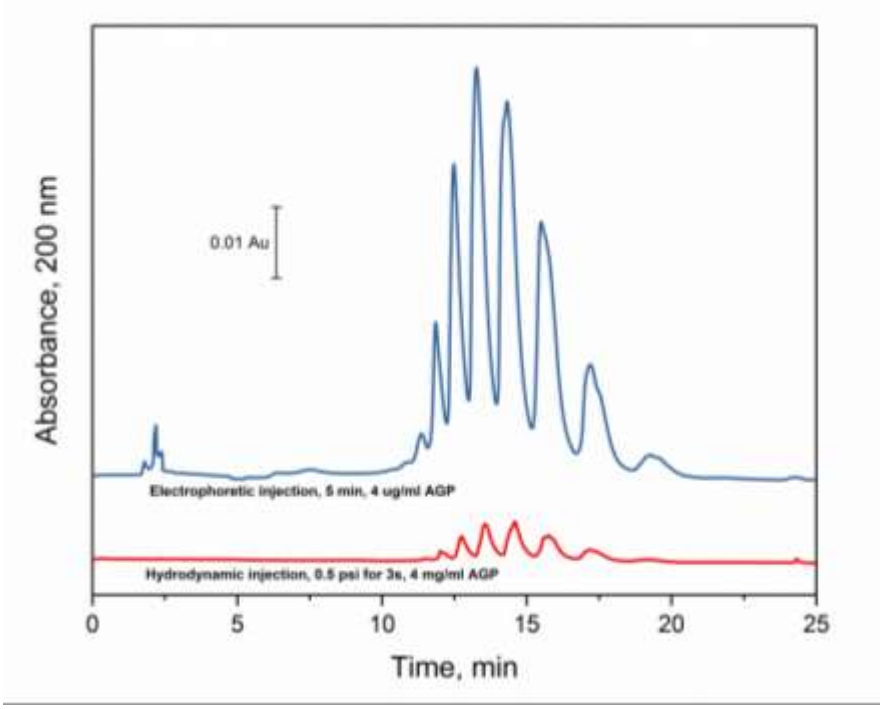

Figure S1. Electropherograms for normal and purified AGP as obtained by electrophoretic injection and hydrodynamic injection. Conditions: separation voltage, -30 kV; capillary temperature, 25º C; running buffer, 20 mM acetate buffer (pH 4.2) containing 0.05% PEO and 0.1% Brij 35. The capillary conditioning procedure is given in Section 2.2.

The relative composition profile for the glycoform bands that was seen with these two injection methods was equivalent at the 95% confidence level, as was demonstrated in Figure 4 and discussed in the main body of the text. However, absolute heights and peak areas were much larger under the conditions that were used for electrophoretic injection. There was also a small shift in the observed migration times for the glycoform bands, with these values being an average of 0.2 min shorter when using electrophoretic injection than hydrodynamic injection. This latter effect occurred because the stacked sample during electrophoretic injection could still migrate at a slow rate during the injection process (i.e., an effect that was amplified when using a long injection time (e.g., 5 min).

Comparison of AGP glycoform patterns using various pretreatment methods

Figure S2 compares the glycoform patterns that were obtained for a commercial sample of normal AGP (i.e., as isolated and purified by the manufacturer through a series of chromatographic steps) and AGP which had been obtained from normal pooled human serum after pretreatment with both acid precipitation and desalting or desalting alone. The two sets of pretreatment methods generally gave similar results for the AGP serum sample; the only exception in this case was that desalting alone giving a slightly higher apparent band at about 21 min, which is believed to be due to minor contamination from another protein with a low pI value in the sample. In addition, both of these methods gave isolated AGP samples that had a slight shift in the intensity of their bands with higher migration times when compared to the commercial samples of normal AGP. As stated in the main body of the text, this shift is probably related to the different approach that had been used by the manufacturer to pretreat and purify the commercial AGP standard.

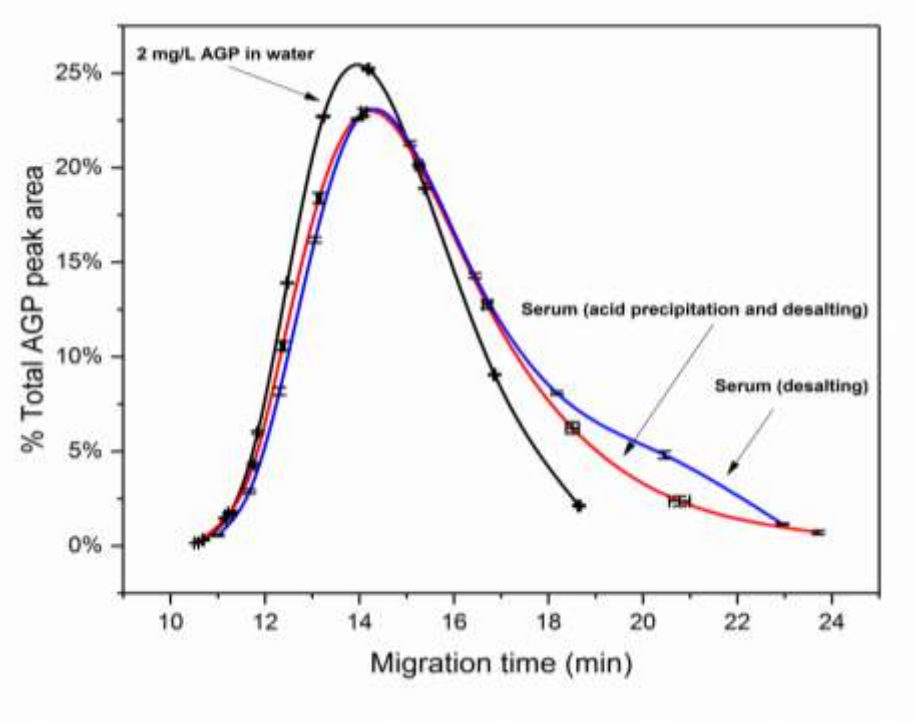

Figure S2. Glycoform pattern obtained for a commercial sample of normal and purified pooled AGP or AGP that had been obtained from normal, pooled human serum by acid precipitation plus desalting or using desalting alone. The CE separation and injection conditions were the same as used in Figure 5. The error bars represent a range of ± 1 S.D. for the mean (n = 3).

Comparison of normal AGP and AGP from SLE serum

An expanded view for some of the results in Figure 5 and 6 is shown in Figure S3 on the following page for the electropherograms that were obtained with the purified sample of normal AGP and AGP that was obtained from SLE serum sample 1 or normal pooled serum. As was stated in the main body of the text, the SLE serum gave a glycoform pattern that appeared to have an additional band at a higher migration time (and at a lower charge) than was seen for the bands from normal and purified AGP (Note: As shown in this expanded view, a trace amount of AGP may also have been present in such a band for normal serum).

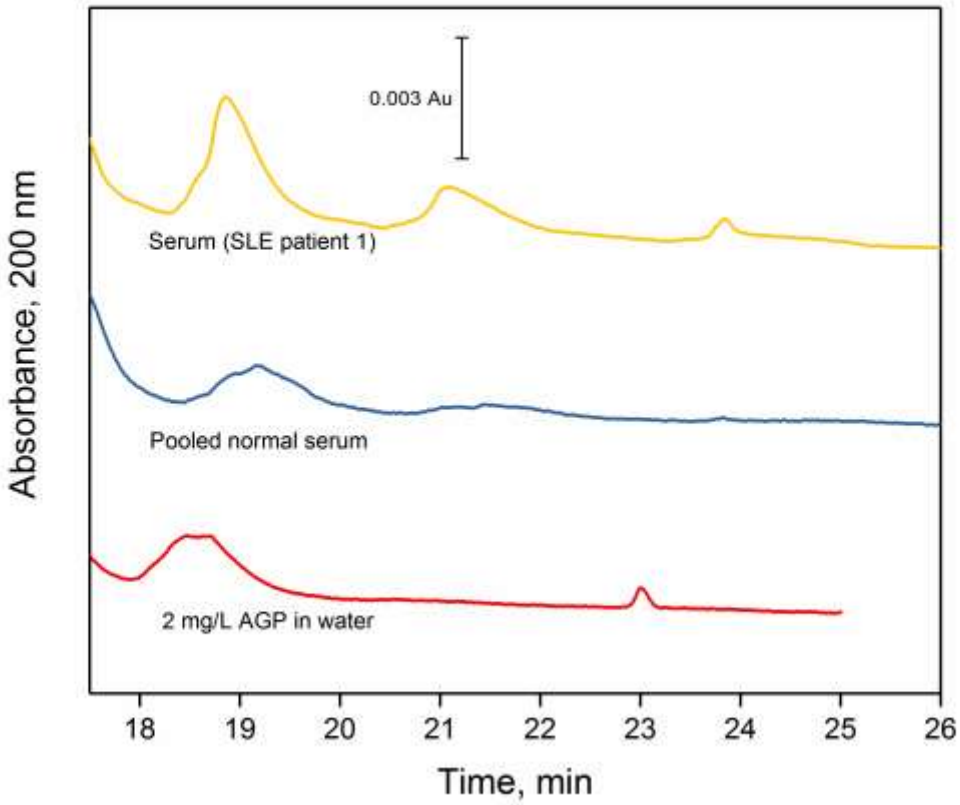

Figure S3. Expanded view of electropherograms for AGP glycoform bands from SLE serum 1 or pooled normal serum (Figure 6) and from normal purified AGP (Figure 5). The band seen at about 24 min in the SLE serum had a migration time that was not equivalent (at the 95% confidence level) to the peak seen at 23 min in the aqueous sample of purified AGP.