Monitoring the Effects of Ethanol and Sucrose on Thermal Stability of Bovine Serum Albumin (BSA) Using the Nanoplus HD

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Abstract.

The effects of sucrose and ethanol on the thermal stability of bovine serum albumin (BSA) are studied with dynamic light scattering using the NanoPlus HD. The change in size of the BSA monomer is observed as an indicator of its melting point, or denaturing of the protein. The addition of 20% sucrose stabilizes the thermal melting point of BSA by ~2°C, while the addition of food-grade ethanol destabilizes the thermal melting point by ~4°C. The NanoPlus HD from Particulate Systems is suitable for monitoring the thermal stability of proteins.

Introduction

Albumin is the most abundant protein in plasma and is responsible for keeping fluids from leaking out of blood vessels, nourishing tissue, and transporting various substances including drugs, hormones, vitamins and others throughout the body. In this study, we examine Bovine serum albumin (BSA) which is homologous with human serum albumin in reactions with commonly ingested substances such as ethanol and sucrose. The BSA monomer is also a macromolecule with a hydrodynamic diameter of about 7-8 nm. The NanoPlus HD has a measurable size range of 0.1 nm to 12.3 µm and a controlled temperature range of 0° to 90°C which fits the ranges of the analysis.

Materials and Methods

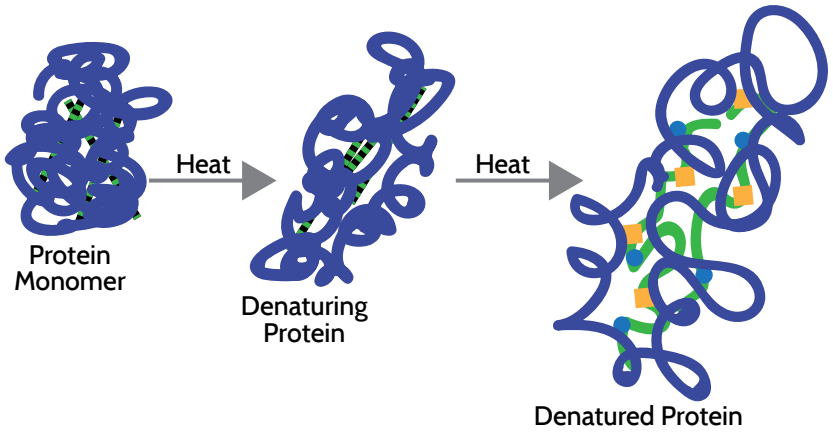
Materials

- Bovine serum albumin, (Thermo Scientific), (Lot# PL209216)
- Filters, 0.02 µm, (GE Healthcare), (Lot# G9887261)

Method

An aliquot of a 2 mg/mL bovine serum albumin (BSA) suspension is used for each test. Ten drops of water, food-grade ethanol, and 20% sucrose in water are added to each aliquot to alter the environment of the BSA. A 0.02 µm filter was used to filter each solution before analysis to ensure that no aggregates interfere with the particle size measurement.

The measurement parameters in the intuitive NanoPlus HD software were set to take size measurements at temperature intervals of 5° C from 25° to 50° C then at intervals of 1° C from 51° to 90° C. A size exclusion of 100 nm was set prior to analysis in order to prevent gas bubbles formed during the temperature increase of the sample preparation from interfering with the measurements. The glass cell was used and the number of accumulations was set to 10.



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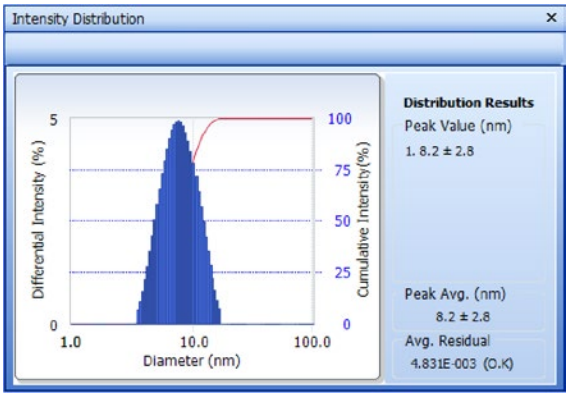

Figure 1. Size of the BSA in water at 25°C.

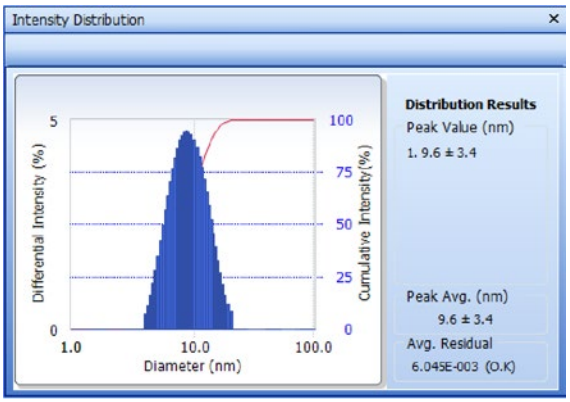

Figure 2. Size of the BSA in food-grade ethanol at 25°C.

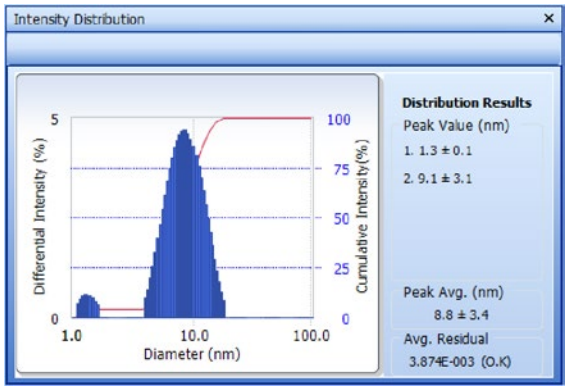

Figure 3. Size of the BSA in 20% sucrose at 25°C. The additional peak is caused by a sucrose molecule, indicating the high sensitivity of the NanoPlus HD.

Results

The BSA monomers in the solution containing distilled water exhibit diameters of approximately 7.9-8.4 nm. When the cell reached 68.4° C, the particles began to grow in size, reaching 8.6 nm and continually getting larger until the cell reached 90.4° C, at which point the monomers were approximately 60.3 nm in diameter.

Before being heated, the BSA monomers in the solution containing ethanol are larger in size, beginning at 9.4 nm in size, most likely because ethanol slightly denatures the protein. The monomers exhibit diameters of approximately 9.7-10.3 at temperatures less than 64° C. At 64.3° C, the monomers began to grow in size, reaching 11.3 nm in diameter and continually getting larger as the cell reached 90.3° C, at which point the monomers are approximately 72.7 nm in diameter.

The BSA monomers in the solution containing sucrose exhibit diameters of approximately 8.6-9.3 nm in at temperatures less than 70° C. When the cell reached 70.4° C, the monomers began to grow in size, reaching 9.8 nm and continually getting larger as the cell reached 90.3° C, at which point the monomers are approximately 39.0 nm in diameter. Figure 3 to the left shows an additional small peak around 1 nm. A solution containing the 20% sucrose solution and no BSA was then analyzed. The results show a repeatable size distribution at about 2 nm (Figure 4) and what appears to be noise at 1.3 nm in Figure 3 is actually particles in the sample and possibly the sucrose molecule since it is not present in the water or ethanol additions. The water source was checked for contaminants as well and no significant scattering intensity or repeatable size distribution was observed.

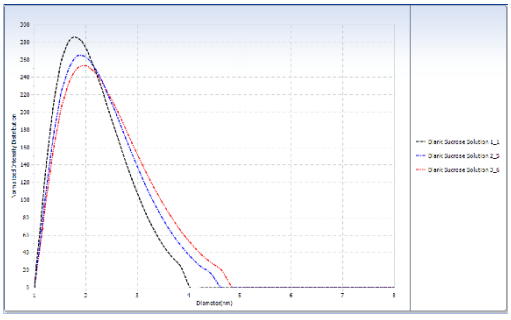

Figure 4. The above temperature gradient graph shows how each sample changed in size.

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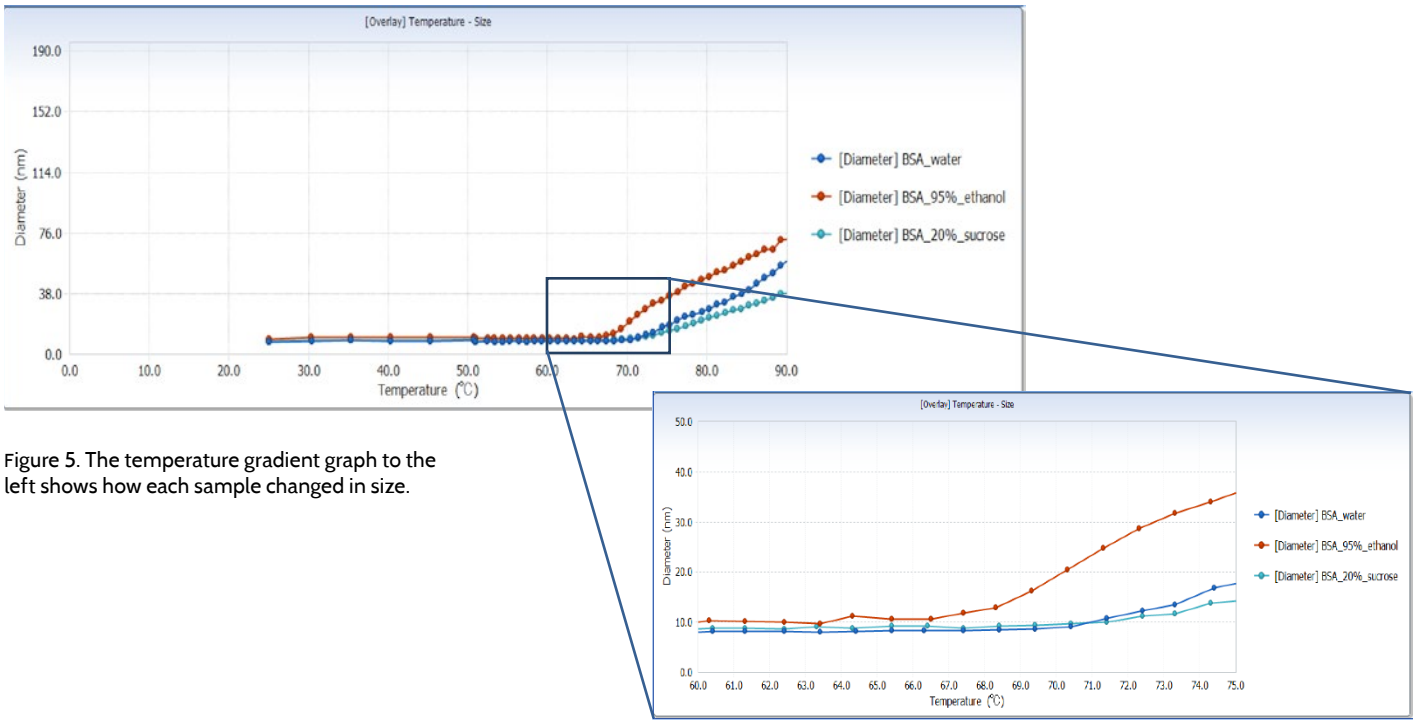

Figure 5. The temperature gradient graph to the left shows how each sample changed in size.

Conclusion

The addition of food-grade ethanol destabilizes the thermal melting point of BSA by approximately 4° C, while the addition of 20% sucrose in water stabilizes the thermal melting point of BSA by approximately 2° C. The NanoPlus HD from Particulate Systems is suitable for monitoring the change in protein monomer behavior at different temperatures and in different additive environments. In addition, the high sensitivity of the NanoPlus HD allowed for the determination that sucrose will appear in a size distribution and should not be assumed to be noise. The NanoPlus HD software is intuitive and reports data in a publication-ready format.

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