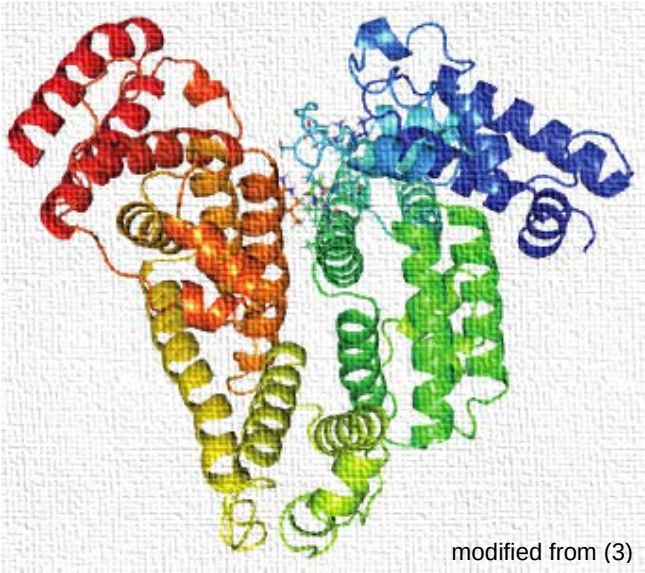
Dimerization of Bovine Serum Albumin As Evidenced By Particle Size and Molecular Mass Measurement

Relevant for: DLS, SLS, Litesizer™, protein, oligomerization, denaturation, buffer

Preparation methods have a large influence on the folding and oligomerization behavior of proteins in solution. Here we compared bovine serum albumin (BSA) dissolved in deionized water (BSA-H_2O) to BSA resuspended in the isotonic buffer PBS (BSA-PBS). Particle size results returned by the Litesizer™ suggested that BSA-H_20 had suffered denaturation and/or aggregation, while BSA-PBS primarily consisted in native BSA dimers. Molecular mass measurements performed on BSA-H_20 returned erroneous results due to the multimodal nature of the sample, while those performed on BSA-PBS displayed the expected molecular mass of dimeric BSA. This indicates that the Litesizer™ is a useful tool for the optimization of sample preparation and the quality control of proteins.



modified from (3)

1 Introduction

Bovine serum albumin (BSA) is a small, stable and moderately non-reactive protein which is commonly used in the lab as protein concentration standard, as blocker in a variety of immunoassays or as cell culture supplement. Being the most abundant serum protein in bovine blood, which is a byproduct of the cattle industry, BSA is a relatively cheap and easily accessible compound.

According to the literature, BSA has a nominal size of 7.1 nm and a molecular mass of 66.5 kDa (1). Bovine serum albumin shows a natural tendency to dimerize under stress conditions, with BSA dimers displaying a molecular mass of 132 kDa (2).

Phosphate-buffered saline (PBS) is a water-based salt solution which is isotonic to mammalian cells and stabilizes the pH at neutral values (commonly pH 7.4). This buffer is widely used to dissolve proteins, resuspend cells or as transport solution for biological samples.

In order to highlight the influence of sample preparation methods on the structure of the solubilized protein, we dissolved purified BSA either in deionized water or in PBS. We then compared the particle size and molecular mass values returned by the Litesizer™ particle analyzer for the two BSA solutions.

2 Experimental Setup

Two different BSA stock solutions were prepared.

Sample 1 consisted in BSA (≥ 96 %, obtained by cold ethanol fractionation, Sigma-Aldrich, Vienna) dissolved in 0.2 µm-filtered deionized water.

Sample 2 consisted in BSA from the same source dissolved in PBS (0.01 M, pH 7.4, also from Sigma-Aldrich).

Four different concentrations (8, 4, 2 and 1 mg/mL) were prepared for each sample. The measurements were performed on a Litesizer™ 500, in quartz cuvettes and at 25 °C.

For the particle size measurements, the 8 mg/mL samples were used. The number of runs, the measurement angle as well as the filter and focus position were determined automatically by the instrument.

The molecular mass measurements were performed in side scatter using a dn/dc ratio of 0.185 mL/g. The four different BSA concentrations were used to generate the Debye plot. Toluene was used as reference.

Both size and molecular mass measurements were performed in triplicate.

3 Results and Discussion

3.1 Particle Size Results

The Litesizer performs dynamic light scattering (DLS) to calculate the hydrodynamic diameter of suspended particles. As shown in Figure 1, BSA dissolved in water showed a bimodal particle size distribution, as two well-defined peaks were visible in the intensity-weighted size distribution curve. While the first peak corresponded to a mean particle size < 3 nm, much lower than the expected 7 nm size of the BSA monomer, the second peak had a mean size of about 22 nm, which was largely above the expected size of the BSA dimer. Thus, it appeared that neither BSA monomers nor BSA dimers could be observed in this sample. This suggested that some level of denaturation and/or aggregation had occurred.

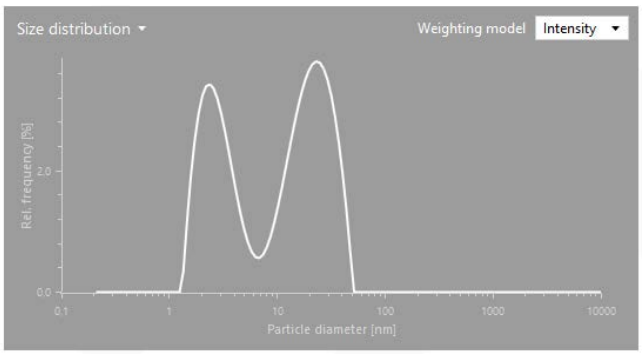


Figure 1 - Particle size distribution by intensity of BSA dissolved in deionized water

In contrast, the DLS results for the BSA sample dissolved in PBS showed a clearly monomodal size distribution (Figure 2). With an average particle size of 12.4 nm (see Table 1), the result suggested that the BSA solution in PBS consisted predominantly of BSA dimers.

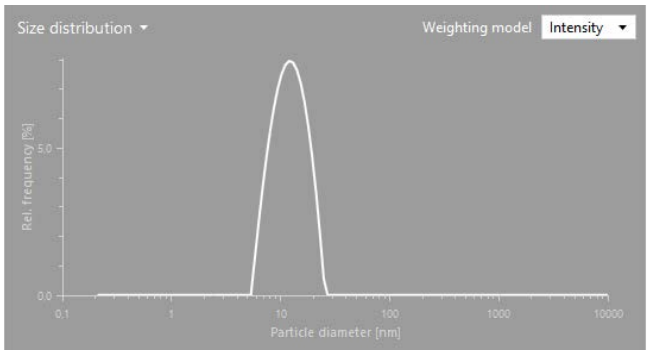


Figure 2 - Particle size distribution by intensity of BSA dissolved in PBS

3.2 Molecular Mass Results

Molecular mass results returned by static light scattering (SLS) are not derived from the scattering intensity of each individual size present in the sample, but from the scattering intensity of the overall size distribution. Hence, SLS is only meaningful when performed on monomodal samples.

Molecular mass calculations performed on the BSA sample dissolved in H_20 returned an average molecular mass of 56.8 kDa (Figure 3, Table 1). While this result can seem consistent with the expected molecular mass of monomeric BSA, the DLS results indicate that the sample is bimodal (see section 3.1). Therefore, the molecular mass result constitutes an “average” value for the two particle species present in the sample rather than the molecular mass of BSA monomers, and should be considered as erroneous.

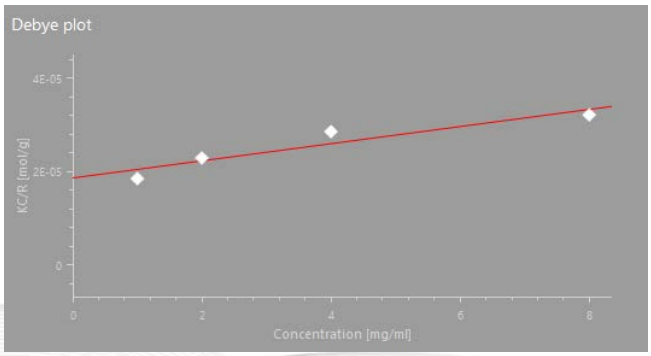


Figure 3 – Debye plot for BSA suspended in deionized water

As DLS results indicate that BSA dissolved in PBS is monomodal, we can assume that the molecular mass results returned by SLS for that sample are meaningful. As shown in Figure 4 and Table 1, this sample displayed a mean molecular mass of 141 kDa, consistent with the expected molecular mass of BSA dimers (theoretical mass: 132 kDa). This confirmed the observation made by DLS that BSA had indeed to a very large extend dimerized when dissolved in PBS.

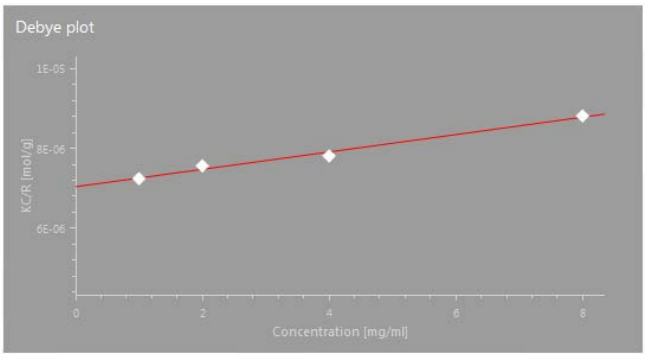


Figure 4 – Debye plot for BSA suspended in PBS

Solvent	Peak by Intensity [nm]	Polydispersity Index [%]	Molecular Mass [kDa]
H_2O	22.50	29.1	59.1
H_2O	22.84	27.6	57.7
H_2O	21.97	23.4	53.6
PBS	12.65	15	144
PBS	11.60	20.7	139
PBS	13.09	22.4	142

Table 1 - Comparison of particle size and molecular mass results of BSA dissolved either in deionized water or in PBS

4 Conclusion

When BSA was dissolved in deionized water, DLS results showed a bimodal size distribution in which neither monomeric nor dimeric BSA particles could be identified. This suggested that the dilution of the BSA in a hypotonic and not pH-stabilized solvent led to a denaturation of BSA molecules and an aggregation of the denatured monomers. Due to the multimodal nature of the sample, molecular mass measurements performed on this dispersion returned erroneous results.

In contrast, BSA dissolved in the isotonic and pH-neutral buffer PBS displayed a monomodal distribution, with an average particle size consistent with that of BSA dimers. The fact that BSA dissolved in PBS spontaneously assembled into dimers was further confirmed by molecular measurement results, which returned average values very close to the expected mass of dimers.

This report highlights the fact that the preparation method used to solubilize proteins has a crucial influence on protein folding and oligomerization. In our case, although the manufacturer of the BSA stated that the product could be resuspended in water, our data indicate that this led to denaturation and/or aggregation of the protein. Resuspension in PBS was preferable, as we could then detect native, dimeric protein both by DLS and SLS.

The Litesizer™ 500 enables the user to measure the particle size and molecular mass of protein samples and even to obtain information about the tertiary and quaternary structure of the solubilized protein.

5 References

1. **Squire, Phil G, Moser, Peter und O'Konski, Chester T (1968).** Hydrodynamic properties of bovine serum albumin monomer and dimer. *Biochemistry* 7(12):4261-4272.

2. **Brahma, Amrita, Mandal, Chlabinath und Bhattacharyya, Debasish (2005).** Characterization of a dimeric unfolding intermediate of bovine serum albumin under mildly acidic condition. *Biochimica et Biophysica Acta - Proteins and Proteomics* 2:159-169.

3. **Chinnathambi, Shanmugavel, et al. (2015).** Effect of Moderate UVC Irradiation on Bovine Serum Albumin and Complex with Antimetabolite 5-Fluorouracil: Fluorescence Spectroscopic and Molecular Modelling Studies. *International Journal of Spectroscopy* 2015:1-12.

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