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APPLICATION NOTE

Investigating the Particle Size, Molecular Weight and Thermal Denaturation of Lysozyme

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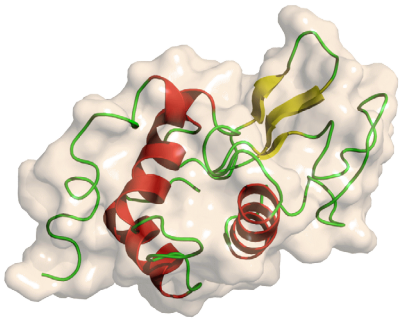
Introduction

Lysozyme, also known as muramidase or N-acetylmuramide glycanhydrolase is abundant in secretions including tears, saliva, human milk, and mucus. It is an antimicrobial enzyme produced by animals that forms part of the innate immune system that plays an important role in the prevention of bacterial infections. It can destroy the cell walls of certain bacteria and thereby act as a mild antiseptic.

Lysozyme is a commonly used enzyme for lysing Gram-positive bacteria. For example, E. coli can be lysed using lysozyme to free the contents of the periplasmic space. Lysozyme was the first enzyme structure to be solved via X-ray diffraction. The primary structure of lysozyme is a single polypeptide containing 129 amino acids. In physiological conditions, lysozyme is folded into a compact, globular structure with a long cleft on the protein surface. The comparatively simple structure and low cost make it a popular model in much current biological research.

The molecular weight of lysozyme is 14.4KDa, which is small, and lysozyme denatures at high temperatures. The scattering intensity of lysozyme is extremely weak and is a challenge for dynamic light scattering (DLS) measurement.



Three-dimensional Structural Lysozyme According to Protein Data Bank

Theory and Instrumentation

DLS measures the intensity fluctuations of the sample due to Brownian motions of particles. The diffusion coefficient D is obtained and related to the particle size, i.e., the hydrodynamic diameter DH, by the Stokes-Einstein equation.

$$D = \frac{k_B T}{3\pi\eta D_H}$$

Where kB is the Boltzmann constant, T is the temperature, and η is the dispersant viscosity.

After obtaining the diffusion coefficient, the molecular weight can be calculated by the empirical Mark-Houwink equation.

$$D = K \cdot M^{-\alpha}$$

Where K and α are constants that relates to the molecular density to some extent.

In this study, the lysozyme sample was characterized by the BeNano 90 Zeta (Bettersize Instruments Ltd.) which adopts a 10mW laser with the wavelength of 633nm. In addition, in the BeNano 90 Zeta, single-model optical fibers are used for signal transmission in order to maximize the signal-noise ratio; high-speed correlators are utilized such that the fast-decay of correlation functions for small particles can be calculated sufficiently.

Experiment

Lysozyme samples with different concentrations were prepared in PBS buffer solution with pH of 7. The sample information is as follows in Table1.

No.	Concentration	Note
1	10 mg/mL	
2	5 mg/mL	
3	30 mg/mL	Protein denaturation study

Table 1. Information of lysozyme sample

The measurement temperature was set to be 25℃ ± 0.1℃ through a built-in temperature control system of the BeNano 90 Zeta. Since lysozyme molecules were very small and the scattering intensity was extremely weak, the presence of impurities such as dust would have a significant impact on the measurement results. Therefore, the samples were filtered by a 220nm filter before the measurement. Each sample was measured at least three times to ensure repeatability of the results.

Results and Discussion

Correlation functions were obtained through the scattered light signals of the samples.

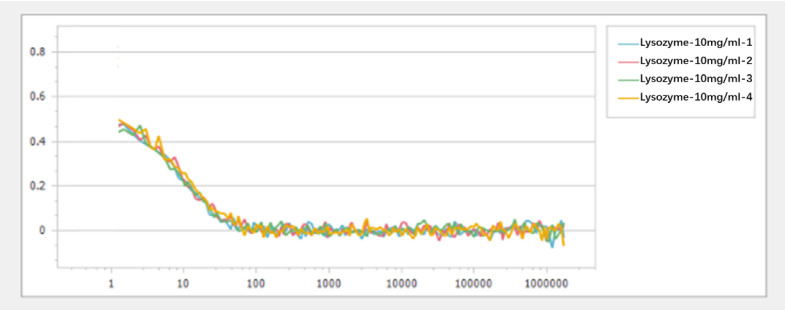


Figure 1. Correlation functions of 10mg/mL lysozyme solution

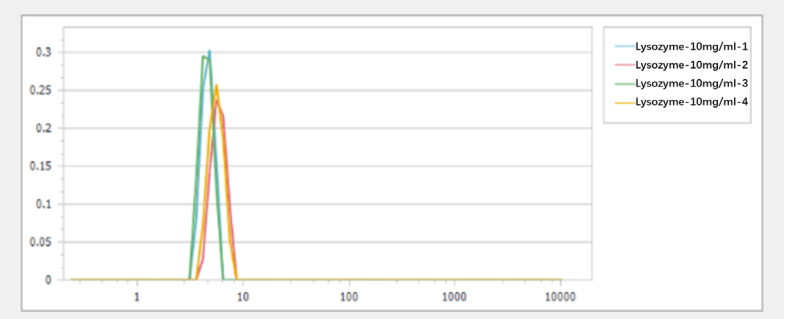


Figure 2. Size distributions of 10mg/mL lysozyme solution

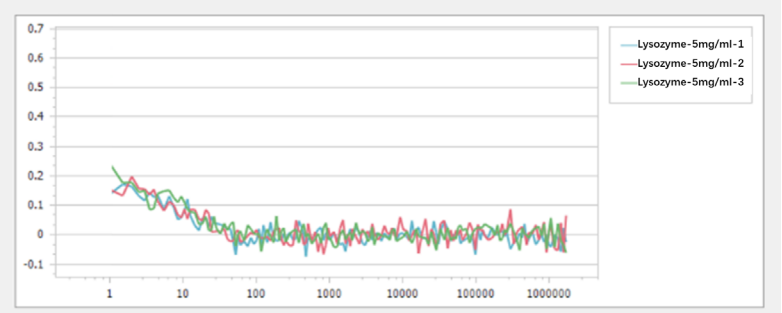


Figure 3. Correlation functions of 5mg/mL lysozyme solution

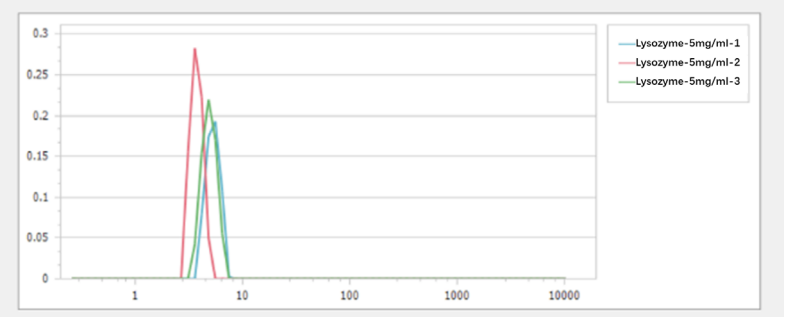


Figure 4. Particle size distributions of 5mg/mL lysozyme solution

As shown in the above Figure 1-4, correlation functions of small particles decayed very fast, due to the rapid Brownian motion. Signal-noise ratios of correlation functions were high enough for a good repeatability, which indicated the excellent sensitivity and stability of the BeNano 90 Zeta. A powerful calculation capability of the BeNano 90 Zeta enables the small particles to have sufficient signals for the correlation function to provide reliable results within a very short time. Results obtained from the multiple measurements are listed below.

As shown in Table 2, the particle size of lysozyme was measured to be between 3 and 4 nm. The molecular weight of lysozyme can be calculated through the Mark-Houwink equation using the K and α constants of lysozyme. It can be seen that the calculated molecular weight of lysozyme (12.6KDa), at the concentration of 30mg/mL, was very close to the theoretical value (14.4KDa) of lysozyme.

Concentration	Z-ave (nm)	Calculated Molecular Weight (Da)
10 mg/mL	3.47±0.31	10.3 K
5 mg/mL	3.79±0.90	12.4 K
30 mg/mL	3.63±0.22	12.6 K

Table 2. Particle sizes of lysozyme at different concentrations at 25℃

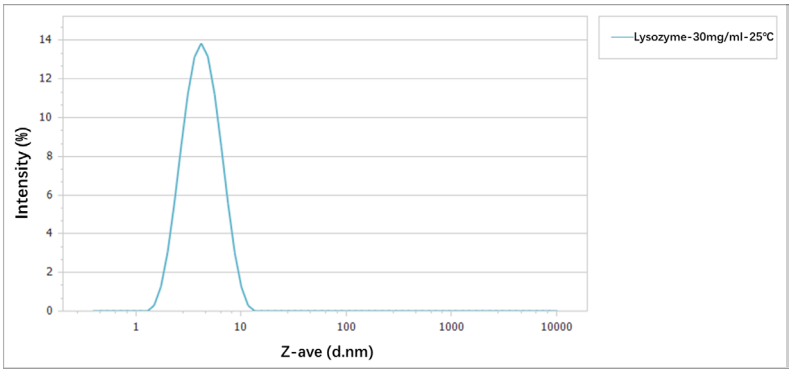


Figure 5. Size distribution of 30mg/mL lysozyme at 25℃

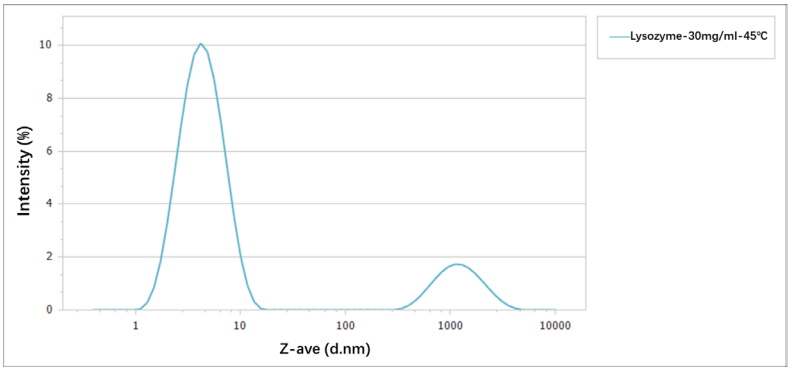


Figure 6. Size distribution of 30mg/mL lysozyme at 45℃

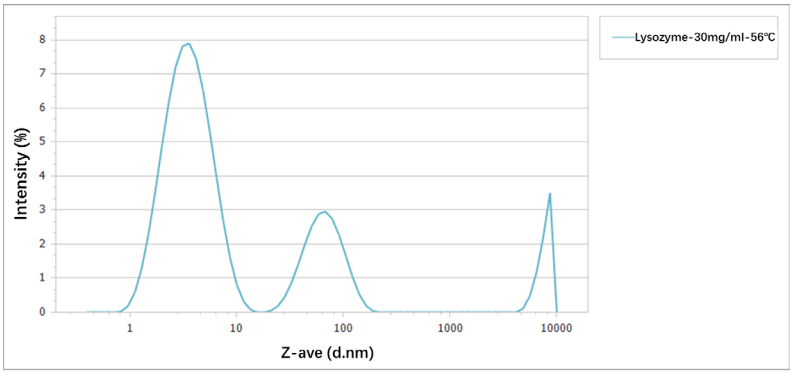


Figure 7. Size distribution of 30mg/mL lysozyme at 56℃

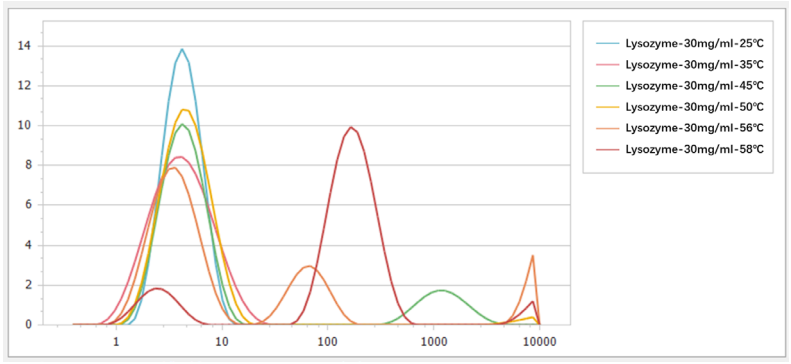


Figure 8. Size distributions of 30mg/mL lysozyme at different temperatures

As can be seen in Figure 5-8, the particle size of lysozyme was initially small and had a narrow distribution at room temperature（25 ℃）, whereas large lysozyme aggregates were formed due to protein denaturation at high temperatures. Figure 9 and Figure 10 show that the Z-average mean and scattering intensity of lysozyme were stable at various temperatures below 50 ℃ , while they increased dramatically when temperature exceeded above 55 ℃ , due to a structure change caused by the denaturation of lysozyme at high temperature and consequently the generation of a significant number of aggregates.

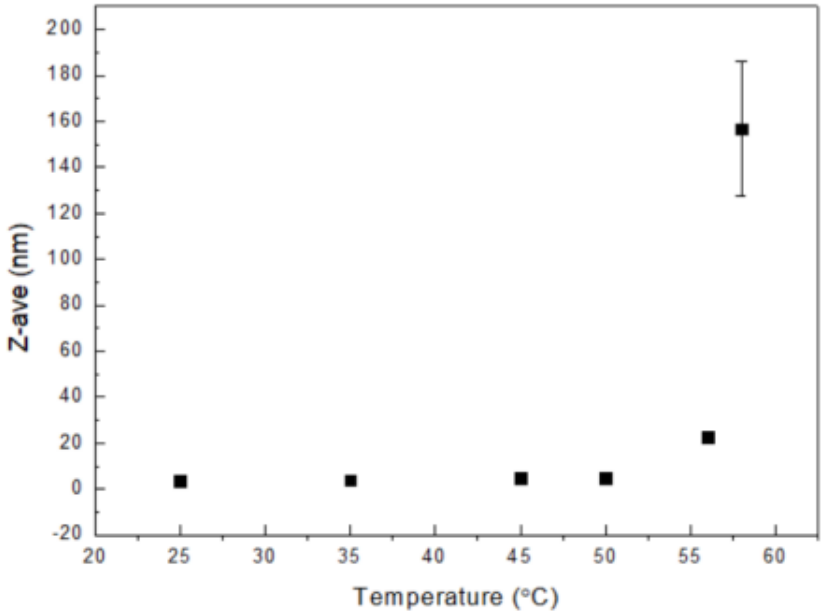


Figure 9. Z-average sizes of 30mg/mL lysozyme at different temperatures

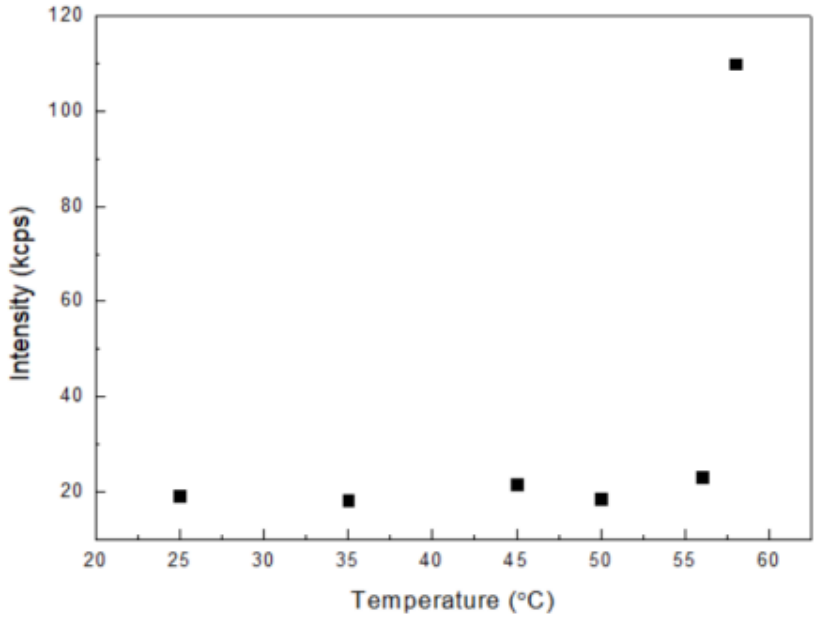


Figure 10. Scattering intensities of 30 mg/mL lysozyme at different temperatures

Conclusion

In this application note, with the BeNano 90 Zeta, the particle size of lysozyme, at the concentration of 30mg/mLm, was measured to be 3.63nm ± 0.22nm and the molecular weight of lysozyme was calculated to be 12.6KDa through the empirical Mark-Houwink equation. The study on the lysozyme denaturation at high temperature has been successfully carried out, by utilizing the precise temperature-control system of the BeNano 90 Zeta.

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