High-Sensitivity Detection of Latex Agglutination by Ultrasound Scattering Techniques

超音波散乱法によるラテックスの凝集の高感度検出

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1. Introduction

The latex agglutination method is a promising technique which enables us to visualize the antigen-antibody reaction through aggregation of antibody-coated microparticles with a small amount of antigen. While optical techniques are commonly used, we have developed a novel technique utilizing ultrasound scattering to achieve detection of aggregation in optically turbid suspensions. In order to enhance the sensitivity to the dilute suspension, a new algorithm was developed by splitting the signal of the aggregation from the total signal. In addition, high-speed sampling of ultrasound pulse was achieved to allow quick detection of the signal for a clinical use.

Before applying the technique to conventional antigen-antibody systems, the avidin-biotin system was employed as a model system for studying latex agglutination since its dissociation constant is expected to be extremely small as schematically illustrated in **Figure 1**. Two-types of microparticles, polystyrene (PS) and silica particles were employed to examine the acoustical sensitivity to the aggregation process. To confirm the presence of aggregated particles, optical images were obtained by a phase-contrast optical microscope.

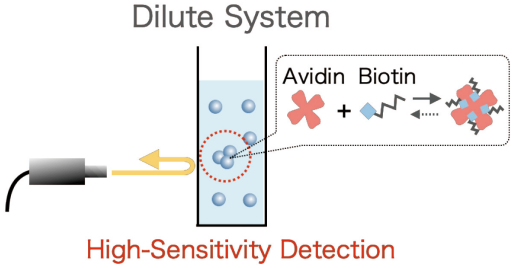

Fig. 1 Schematic representation of measurement of the avidin-biotin latex aggregation using the ultrasound technique.

2. Experimental section

2.1 Sample

Surface modified PS and silica particles were obtained from micromod Partikeltechnologie GmbH. The particles were further coated by biotin (EZ-link NHS-PEG 12 biotin Thermo 21312) and biotin-PEG11-amine (B5565, Tokyo chemical industry, Japan). The particles were dispersed in phosphate buffer (1/15 M, pH 7.2 phosphate buffer, Wako Japan) to obtain 0.1wt% suspension, and their aggregation behavior was investigated by adding prescribed amount of avidin (nacalai tesque, Japan). The avidin concentration c_{avi} was varied in range 0.05 – 0.0003125 mg/ml. The nominal particle diameters of the PS and silica particles were respectively 200 and 220 nm.

2.2 Time resolved dynamic ultrasound scattering (TR-DSS)

A spike pulse emitted from BLP12R remote pulser (iSL, Japan) was transferred to a 20 MHz or 30 MHz longitudinal plane wave transducer (KGK, Japan) immersed in a water bath to generate ultrasound pulses. The back scattered signals were received by the same transducer, followed by successive recording with a CSE1622 high-speed digitizer (GaGe, DynamicSignals LLC, Canada). The vertical resolution of the digitizer was 16-bit and the time resolution was 200 Mega samples/s.

The obtained scattered intensities were

corrected using the magnitude of the time correlation function to separate the noise from the data. The pulse repetition time was set to 1 ms to achieve high-speed monitoring of the aggregation process. Thanks to the rapid recording, each sampling time was dramatically shortened from 30 minutes to 20 seconds. Further rapid measurements are attempted to complete acquisition within 0.08 seconds for the practical applications.

3. Results

As reported previously, the TR-DSS technique allowed us to detect the avidin-biotin reaction down to c_{avi} = 0.000625 mg/ml through the aggregation process. The avidin concentration dependence of the aggregation behavior was found to be more prominent for the PS particles than that of silica particles. In order to dig the findings further, particle sizing was attempted by the

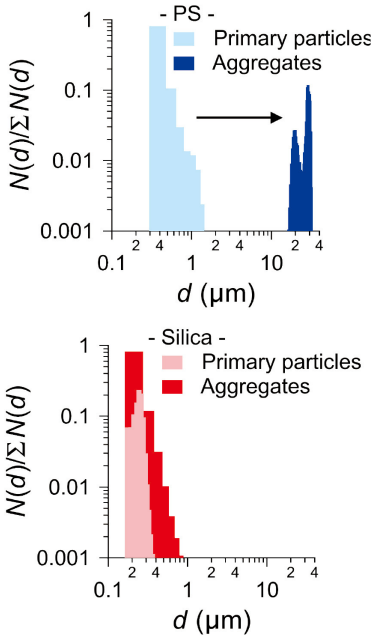

Fig. 2 The normalized size distribution obtained for the biotin coated PS (blue) and the biotin coated silica (red) particles evaluated by the Z-DSS method.

Z-DSS technique, which directly probed the population of aggregates as a function of the diameter. The particle number and the diameter are simultaneously obtained by the individual scattering intensity and the sedimentation velocity. **Figure 2** shows the normalized size distribution obtained for the biotin coated PS and the biotin coated silica particles evaluated by the Z-DSS method. While the formation of aggregates was confirmed for the silica particles, the PS particles showed noticeable increase in the diameter.

The scattering intensity of a single particle by the ECAH theory is illustrated in **Figure 3**. It was recognized that although the intensity of PS nanoparticle is smaller than that of silica at the Rayleigh regime, the intensity of PS is larger than that of silica in the range 20 - 40 μm. Such a scattering profile could account for the high sensitivity detection of the PS latex agglutination.

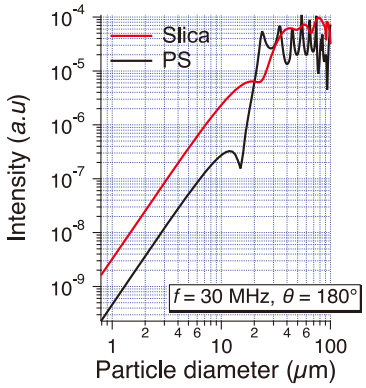

Fig. 3 The particle diameter dependence of the ECAH scattering intensity obtaind for PS (black) and silica (red) particles.

4. Conclusions

We have developed a novel dynamic ultrasonic scattering technique (TR-DSS and Z-DSS) to probe the latex agglutination. As an example, the biotin-avidin system was studied, and the detection of avidin was achieved down to c_{avi} = 0.000625 mg/ml. The PS particles was more suitable to detect avidin-biotin latex agglutination using ultrasonic scattering technique.