3P5-2

Detection of Avidin-Biotin Latex Agglutination using Ultrasound Scattering Techniques

超音波散乱法によるアビジン-ビオチン ラテックス粒子の凝集体解析

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1. Introduction

The latex agglutination method is a convenient technique which enables us to visualize the antigen-antibody reaction though aggregation of antibody-coated microparticles with a small amount of antigen as schematically illustrated in **Figure 1**. 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 overcome the cost-issue as well as its sensitivity to the dilute suspension, a new algorithm was developed to split the signal of the aggregation from the total signal. In addition, high-speed sampling of ultrasound pulse was attempted to allow quick detection of the signal for a clinical use.

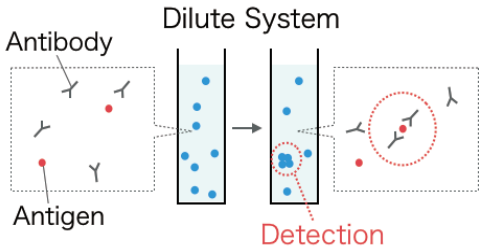

Fig. 1 Schematic representation of aggregation formed in dilute suspension.

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. Two-types of microparticles, polystyrene and silica particles were employed to study the acoustical sensitivity to the aggregation process. In order to confirm the presence of aggregated particles, optical images were obtained by a phase-contrast optical microscope.

2. Experimental section

2.1 Sample

Surface modified polystyrene (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 static/dynamic ultra sound scattering

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 time correlation function to separate the thermal noise associated with the small molecules and/or digital equipment from the signals. Trace amounts of avidin was added to the suspension to monitor the aggregation process. 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.

3. Results

First, avidin (c_{avi} = 0.1 mg/ml) was added to the biotin-coated particle suspension. Prior to the addition of avidin, the measurements have already been started to ensure the background level of the signal. This allowed us to clearly visualized the increase in the small signals associated with direct addition of avidin to the sample (not shown here). Although the increase in the scattered intensity was observed, homogenous mixing of the sample in the holder was more or less limited. Therefore, for the subsequent analysis, biotin-coated particles were carefully mixed outside the sample holder and the measurements were initiated after aging the sample in the thermostat bath.

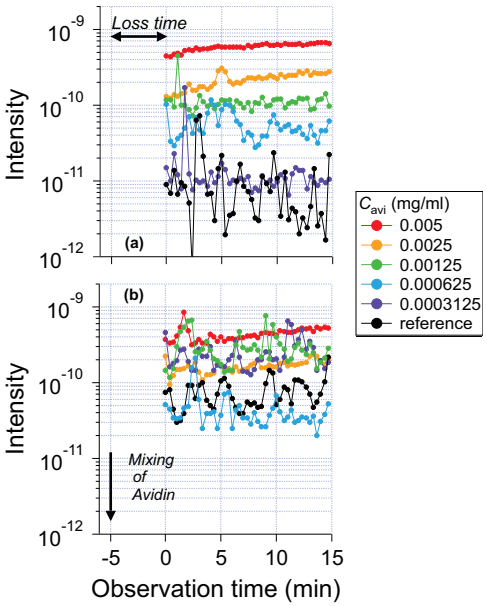

Fig. 2 The time dependence of the scattered intensity obtained for the biotin coated (a) PS and (b) silica particle suspensions in the presence of avidin.

Figure 2 shows the time dependence of the scattered intensity obtained for the biotin-coated (a) PS and (b) silica particles. The new technique allowed us to detect the avidin-biotin reaction up to c_{avi} = 0.000625 mg/ml through the aggregation of biotin coated particles with avidin. It should be noted that the aggregation process was more prominent in the PS suspensions rather than those of silica. This was surprising because the scattering contrast of PS was expected to be smaller than that of silica. The possible mechanism is illustrated in **Figure 3**. Namely, the PS particle coated with biotin has effectively large domain to cause large particle scattering, while those of silica may behave as individual scatterers because of the large density difference.

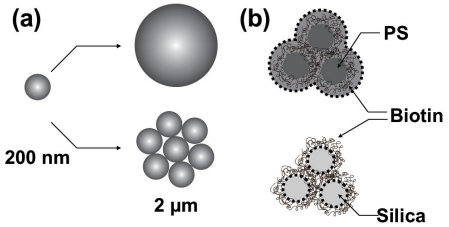

Fig. 3 (a) effective particle size consisting of primaly particles (b) aggregation of biotin coated PS and silica particles. The dotted circle denote the effective size of particle scattering.

4. Conclusions

We have developed a novel static/dynamic ultrasonic scattering technique to probe the latex agglutination. As an example, biotin-avidin system was studied, and the detection of avidin was achieved up to c_{avi} = 0.000625 mg/ml.