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Real-time monitoring of viscosity changes triggered by chemical reactions using a high-speed imaging method

Wooseok Jung, Cedric Hurth, Aimee Ellen Becker, Frederic Zenhausern *

Center for Applied Nanobioscience and Medicine, University of Arizona College of Medicine, Phoenix, AZ 85004, United States

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ABSTRACT

We present a method to monitor in real time peptide self-assembly or polymerization events. The temperature controlled modification of a previously reported splash test setup using high speed imaging enables to observe and measure rheological changes in liquid samples and can, in turn, monitor a peptide self-assembly or polymerization reaction accompanied with specific changes in solution viscosity. A series of 2 mm glass beads were dropped into an Fmoc-L_3-OMe (methylated Fluorenylmethyloxycarbony l-trileucine) solution mixed with Alcalase 2.4 L (EC 3.4.21.62) or first dipped in Tetramethylethylenediamine (TEMED), a catalyst for acrylamide polymerization, then dropped into acrylamide. The resulting splashes were observed using a high speed camera. The results demonstrate that the viscosity changes of the peptide sample during the peptide self-assembly or acrylamide polymerization affect the specific shape and evolution of the splashing event. Typically, the increase in viscosity while the reaction occurs decreased the size of the splash and the amount of time for the splash to reach maximum extension from the moment for the beads to impact the sample. The ability to observe rheological changes of sample state presents the opportunity to monitor the real time dynamics of peptide self-assembly or cross-polymerization.

1. Introduction

There is a constant need in clinical diagnostics to quickly, efficiently and inexpensively characterize a variety of biofluids to allow direct use for bedside monitoring. Viscosity has been shown to be an efficient biomarker in the characterization of multiple biofluids involved in various diseases [1,2]. We have previously developed a setup aimed at recording and analyzing splashing events resulting from the impact of small glass beads [3] and succeeded in showing applications of clinical relevance [4]. This demonstrates a successful application of physical science methods using sample viscosity as a basic criterion for future point-of-care diagnostics applications, while the ability of monitoring viscosity changes directly following the uptake of a particular drug could provide a simple method for measuring its effectiveness for a particular individual patient. Here we report an evolving system for *in situ* characterization of the viscosity using a previously described splash imaging method.

Peptide self-assembly affects the most diverse building blocks in nature and is an inherent property of polypeptides [5]. This phenomenon is useful for many biological applications and innovative technologies [6,7]. The self-assembly of peptides provides a molecular approach for nanostructure production, which can be used for a wide array of medical applications like tissue engineering [8] and 3D cell cultures [9]. The novelty of self-assembling peptides lies in the versatile framework to make other materials, and the ability to manipulate and modify natural resources and involve them in a wide range of processes and applications [10]. Exploiting systems that are self-assembling can not only expand the potential for growth in nanotechnology and medical technology but also demonstrate that peptides in self-assembly configuration can act as structural as well as functional components for recombinant protein production [11]. Meanwhile, self-assembling peptides have shown to be triggered by properties such as pH and enzyme and can lead to gelation of a sample in response [12]. Real-time monitoring of the peptide self-assembly process is a noticeable advance towards practical applications of our method in so far as it analyzes changes in sample states. Being able to utilize that knowledge can provide an easier and more cost-effective approach for biological applications such as characterizing biofluids and taking direct measurements for medical diagnoses. By following the self-assembly model of Fmoc-protected tri-leucine upon de-methylation by Alcalase (Subtilisin A from *Bacillus licheniformis*)

* Corresponding author.
E-mail address: fzenhaus@email.arizona.edu (F. Zenhausern).

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using the viscosity changes in the small (400–600 μL) reaction volume, we were able to employ the splash visualization method to follow a relatively slow kinetic event in real-time with a resolution <1 min.

Another important class of chemical reaction accompanied by large viscosity changes is polymerization. In particular, the polymerization of acrylamide monomers has been extensively studied [13,14] and is widely used in critical bio-analytical separation techniques [15,16]. Upon addition of 1% ammonium persulfate (APS), the chain polymerization of acrylamide monomers is initiated but the addition of TEMED causes side-chain polymerization resulting in a large increase of the polymerization sample viscosity [13]. We were therefore not only able to follow the polymerization reaction using our proposed method but also able to trigger the side-chain polymerization by first dipping the probe beads into a concentrated TEMED solution.

The above illustrations of the possibility to trigger and monitor viscosity evolution of molecular reactions using a simple physical science method open up a wider array of biological or biomedical applications to directly monitor, for instance, the effect of a drug such as warfarin on blood viscosity [17] or potential leukemia drugs with minimal sample preparation restrictions. In addition, the Fmoc-L_3-OMe self-assembly could be used to follow a plethora of other molecular reaction events interfacing with a well-calibrated viscosity evolution using the splash method.

2. Materials and methods

2.1. Peptide dilution and probe preparation

The peptide sample was prepared by mixing 2.0 mg (4.8 μM) Fmoc-L_3-OMe (310205-1, Genscript), 70 μL of 100% Methanol, and 630 μL of 0.1 M phosphate buffer at pH 8 in a 1.5 mL plastic tube. A set of 2 mm spherical glass beads (SiLibeads Type P, Sigmund Lindner GmbH) that were used for the impactor in the splash test was cleaned for 4 min in an O_2 plasma cleaner (PDC-001, Harrick Scientific Corp) to remove any organic pollutant from the surface.

2.2. Splash imaging setup

The existing splash setup previously used for clinical diagnostic of pleural effusions [3,4] was modified by integrating a heater into a sample cuvette. Briefly, the splash imaging setup consists of a vertically-mounted stepped motor, a bead pick-up assembly, and a high speed imaging device described in details elsewhere [3]. The vertically-mounted step motor was set to a drop distance of 95 mm (impact velocity assuming free fall: 3.47 m s^{-1}). All the beads for the measurements were dropped from this same distance and therefore dropped at the same impact velocity. The bead dropping device shown in Fig. 1(b) consists of a notched disk to hold the beads, a step motor to rotate the disk and pick up the beads, a fixed vacuum nozzle to move the beads to the drop area, and a vacuum sensor synchronized with a solenoid valve to drop the bead. An Olympus iSpeed TR camera was used to record the impact at a 10,000 frames/s, whereas a 100 W ultra-high pressure mercury lamp was used as a light source. A plastic cuvette was used as a sample reservoir (volume ~600 μL) and was wrapped with a resistive heating element controlled by a Proportional Integral Differential (PID) feedback loop, and mounted onto and XYZ dovetail positioning stage to ensure that the impact of the bead consistently occurred at the center of the concave meniscus formed by the sample in the cuvette. Fig. 1(a) shows the layout of the setup.

2.3. Peptide self-assembly

The clean glass beads were loaded onto the notched disk via a free-handed vacuumed pick up nozzle. 1.25 μL (1.79 μL/mL) of an Alcalase enzyme (P4860, Sigma–Aldrich) was added to the peptide solution and mixed homogenously. The LabView™ software set the drop height at 95 mm, dropped beads at each measurement interval, and recorded the results. A measurement from 3 beads taken before turning on the heater served as reference data. Then, the heater was immediately turned on to reach 35 °C. Thereafter, 3 bead measurements were taken every minute for a total of 7 sets of measurements including the reference data. The images that were downloaded and collected from the high speed camera were then analyzed to determine the time from impact to maximum splash extension as well as the splash shape.

2.4. Acrylamide polymerization

Clean glass beads were added to the notched disk tray but one slot was filled with concentrated TEMED solution (~99%, T9281, Sigma–Aldrich, Milwaukee, WI). 600 μL of a 2% v/v mixture of bis- and acrylamide solution in a 19:1 ratio containing 1% APS (A3678, Sigma–Aldrich, Milwaukee, WI) was added to the sample vial maintained at room temperature. First, 3–6 clean glass beads were picked up by the vacuum-actuated nozzle and dipped into the TEMED solution before being launched into the sample solution to initiate polymerization. A first set of 3–5 clean bare beads was dropped into the sample solution from a distance of 95 mm to record the reference system state. Then, a set of 3–5 clean bare beads was dropped every 30 s or 1 min interval to probe the solution viscosity for up to 90 min. To prevent excessive evaporation, the sample vial was placed in a machined polycarbonate humidity

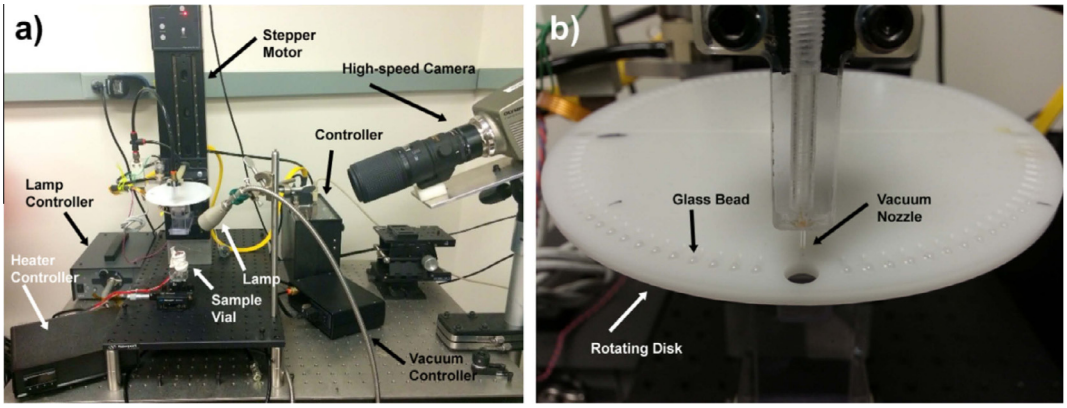

Fig. 1. (a) Splash setup. The sample reservoir cuvette sits directly below the fixed nozzle of the bead dropping device. The high speed camera is placed at a 45° angle to facilitate imaging in good contrast conditions. (b) Detail of the bead dropping for the glass beads.