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Collisional Reaction of Liquid Droplets: Amidation of Dansyl Chloride Observed by Fluorescence Enhancement

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The collisional reaction of droplets is observed by time-resolved fluorescence spectroscopy. Colliding droplets of the reactant solutions are irradiated with a pulsed laser, and the resulting fluorescence spectra and images of the colliding droplets are observed as a function of the elapsed time from the collision. The amidation reaction of dansyl chloride with isopropylamine is observed through fluorescence enhancement on a microsecond time scale. The present method enables us to measure the early stages of reactions in solutions.

The kinetics of chemical reactions between two species in solution can only be investigated by mixing two solutions because the two reactants must be separated before the reaction to be able to set a reaction start time. Kinetic measurements can then performed by observing the time evolution of the concentrations of the species of interest in the solution. Rapid mixing of the two solutions is particularly important for kinetic measurements of fast reactions to ensure that the solution is homogeneously mixed on a time scale shorter than that of the reaction.

The stopped-flow method is commonly used to observe the reaction between two species in solution, whereby the two reactant solutions are delivered to an observational cell through a mixer, and the composition of the reactant and/or product species are analyzed. Kinetic data are obtained by this method because the reaction time corresponds to the distance from the mixer to the analysis point. This method has been applied to various reactions, such as the association of two species and protein folding.[1] However, the stopped-flow method typically suffers from an initial dead time of ca. 1 ms, which is required to obtain homogeneous mixing of the two reactant solutions. Recent technical improvements have reduced the dead time to tens of microseconds.[2–5]

Liquid droplets can also be employed for the measurement of chemical reactions between two reactants in solution, such as the reaction between two trapped reactant droplets in a liquid[6] and a droplet falling on the surface of a reactant solution.[7] Aerosol droplets have also been used to investigate chemical reactions. Simpson et al. have observed chemical reactions during the merging of two droplet streams of H_2SO_4 and NaOH aqueous solutions by Raman spectroscopy.[8–10] However, it is likely that microscopic analysis of the droplets will yield more detailed information on the reaction.

We have also investigated the collision dynamics of liquid droplets. In our previous study, we observed the temporal sequence of the collision of water and ethanol droplets stroboscopically, and observed the appearance of a characteristic protrusion upon collision.[11] We then observed the Raman spectra of colliding droplets at various times after collision and elucidated the mechanism of protrusion formation by deducing the composition of the protrusion from the Raman spectrum.[12]

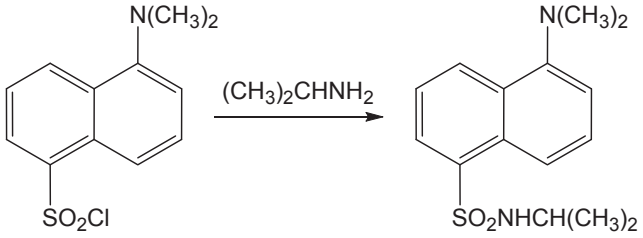

Figure 1. Amidation reaction of dansyl chloride.

Most recently, we measured the coloring reaction of phenolphthalein induced by the collision of droplets of phenolphthalein in ethanol and aqueous NaOH.[13] Through these studies, the developed laser spectroscopic technique has proved particularly useful for the investigation of the collisional reaction of droplets.

In the present study, we applied this technique to observe the amidation reaction of dansyl chloride (DNS-Cl) (Figure 1). DNS-Cl reacts with primary amines, amino acids, and proteins to produce dansyl amide (DNS-amide), which fluoresces more intensely than the reactants. This fluorescence can be used for the quantitative analysis of the reactant and product species.[14–18] In the present research, we investigate the initial kinetics of the amidation reaction induced by the collision of droplets of DNS-Cl in ethanol solution (DNS-Cl/EtOH) and isopropylamine (IPA) droplets. Kinetic measurement within 20 μs is achieved by this method.

A detailed description of the droplet-collision apparatus has been reported previously.[11–13] The apparatus and the experimental procedures employed in the present study are described briefly as follows. The apparatus was constructed around a microscope, which was used to observe droplets tens of micrometers in size. Droplets were produced from reservoirs of the sample solutions using a set of piezo-driven nozzles (Microdrop, MD-K-130), which were triggered independently by electric pulses supplied from a pulse generator. A white light-emitting diode (LED) was used as a strobe light to aid the imaging of droplet collision. The LED was mounted under the collision region and thus illuminated the colliding droplets from beneath. The objective lens of the microscope above the collision region focused the light for imaging. The duration of the LED pulse was set to 1 μs, which was the time resolution of the image measurement. The pulse generator used to trigger droplet generation was also synchronized with the LED pulses with a variable delay. A series of droplet-collision images were recorded by changing the LED timing with respect to droplet generation. The images in the series show different droplets; however, because of the sufficiently small variation between the droplets generated, they show the collision dynamics of the droplets. In the present study, the impact parameter was set close to zero; thus, the colliding droplets had cylindrical symmetry

aligned along the droplet-to-droplet axis throughout the collision. We employed 1.9 mM DNS-Cl/EtOH and neat IPA as the reactants. Conventional fluorescence spectra of DNS-Cl/EtOH and the mixture of DNS/EtOH and IPA were measured by a spectrofluorimeter (JASCO, FP6500).

A pulsed laser and CCD spectrometer were introduced to the droplet-collision apparatus to collect the spectra and the corresponding images of fluorescence emerging from the colliding droplets. Droplets of DNS-Cl/EtOH and IPA (or DNS-Cl and DNS-Cl) were subjected to collision and irradiated by the third harmonic of a Q-switched Nd:YAG laser (Spectra Physics, INDI-40-10) for fluorescence excitation. The colliding droplets were irradiated with a laser beam focused through the same objective lens (Mitsutoyo, M Plan Apo NUV 20×) as the image observation. The objective lens was used because it can sustain the high-intensity laser beam. The size of the focal spot of the laser (ca. 15 μm) was measured from an image taken under irradiation of a quartz plate in the focal region. The laser power was set to ca. 25 μJ pulse^{-1}. The focal position of the laser was adjusted so as to irradiate the edge of the mixing region of the colliding droplets. Fluorescence emerging from the colliding droplets was collected by the objective lens, passed through a dichroic mirror, which reflected the third harmonic of the Nd:YAG laser and transmitted light of longer wavelengths, and divided into two components using a 70% reflection mirror. The transmitted light was then focused onto the CCD of a camera to view the fluorescence images and the reflected light was guided to a CCD spectrometer, constructed in-house, to record the fluorescence spectra. The spectral resolution of the spectrometer was ca. 0.77 nm, which was measured from the width of the spectral line of Ne. The Raman images and the corresponding spectra were simultaneously recorded with each laser shot.

Figure 2 shows a collision sequence of droplets of 1.9 mM DNS-Cl/EtOH and IPA. An oblate-shaped contact region is formed between the two droplets after collision, which we denote as the mixing region. The remaining regions of the original two droplets diminish and completely merge within 20 μs from the collision. An almost identical morphology change is observed for droplet collision of DNS-Cl/EtOH and DNS-Cl/EtOH, as shown in Figure 3. Hence, we can regard the two collisions as the reactive collision and its reference. The concentration of DNS-Cl was set to 0.95 mM in the latter case, in order to equalize the DNS concentration in the colliding two droplets for the two collisions.

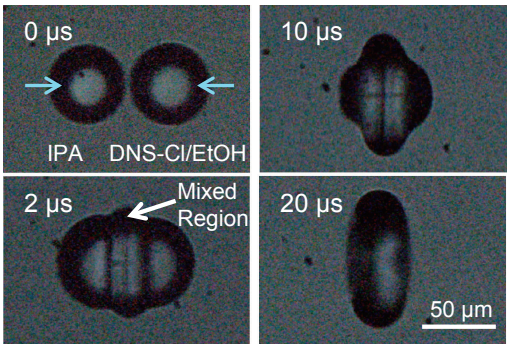

Figure 2. Collision sequence of droplets of 1.9 mM DNS-Cl/EtOH and IPA.

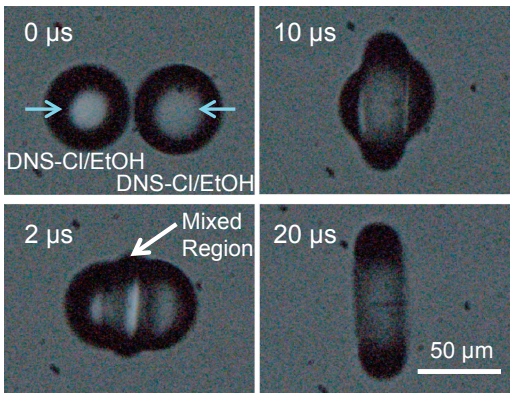

Figure 3. Collision sequence of two droplets of 0.95 mM DNS-Cl/EtOH.

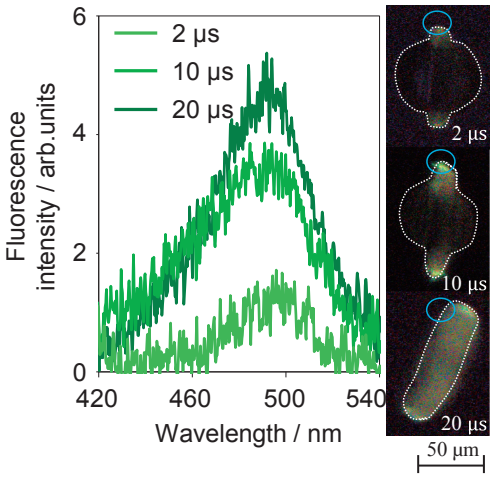

Figure 4. Fluorescence spectra and corresponding images of the mixing region of colliding DNS-Cl/EtOH and IPA droplets. The blue circles in the images indicate the region of laser irradiation. The dotted white curves indicate the peripheries of the colliding droplets for clarity.

Figure 4 shows fluorescence spectra and the corresponding images of the mixing region of colliding DNS-Cl/EtOH and IPA droplets at different elapsed times after collision. The fluorescence spectral peak at 500 nm can be assigned to DNS-amide and/or DNS-Cl. The fluorescence intensity increases with elapsed time up to 20 μs from collision. This result indicates that the fluorescence originates from DNS-amide and that the amidation reaction occurs within 2–20 μs from collision.

Figure 5 shows the fluorescence spectra and the corresponding images of the mixing region of the colliding DNS-Cl/EtOH droplets at different elapsed times after collision. No significant fluorescence is observed in either the spectra or the images. Therefore, we can conclude that the spectra observed in Figure 4 originate from the reaction product, DNS-amide.

Figure 6 shows the integrated intensity of the fluorescence spectra of colliding droplets of DNS-Cl + IPA and DNS-Cl + DNS-Cl as a function of the elapsed time from collision. The fluorescence intensity increases with increasing elapsed time for the collision of DNS-Cl and IPA droplets. This indicates an increase in the concentration of the DNS-amide product in the

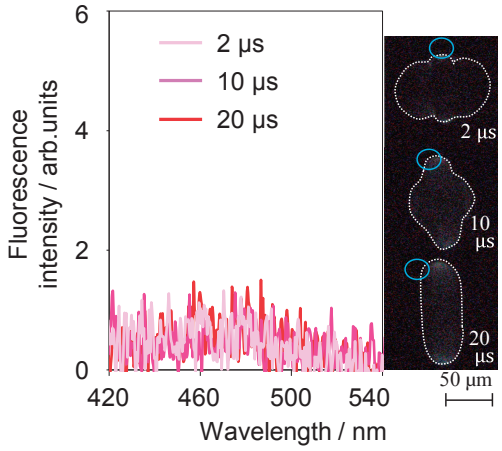

Figure 5. Fluorescence spectra and corresponding images of the mixing region of colliding DNS-Cl/EtOH droplets. The blue circles in the images indicate the region of laser irradiation. The dotted white curves indicate the peripheries of the colliding droplets for clarity.

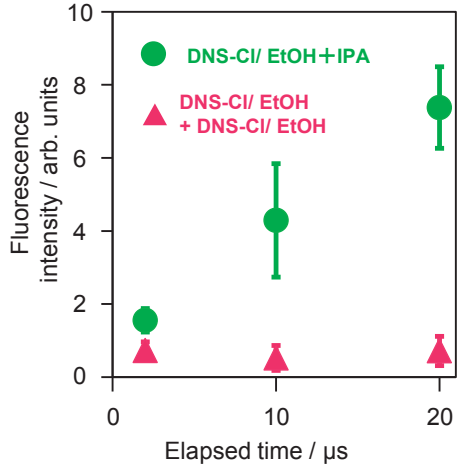

Figure 6. Fluorescence intensities of the colliding droplets of DNS-Cl + IPA and DNS-Cl + DNS-Cl as a function of the elapsed time from collision.

contact region. In contrast, the fluorescence intensity remains constant and relatively low for the collision of DNS-Cl and DNS-Cl droplets.

The fluorescence spectrum of the DNS-Cl/EtOH and IPA mixture recorded by the spectrofluorimeter was ca. 150 times more intense than that of the DNS-Cl/EtOH solution owing to the presence of DNS-amide in the mixture, where DNS-amide has a higher fluorescence quantum yield than does DNS-Cl. As seen in Figures 3 and 4, fluorescence enhancement is observed in colliding droplets of DNS-Cl/EtOH and IPA, which is indicative of the amidation reaction of DNS-Cl with IPA.

The reaction is considered to take place in the contact area of the colliding droplets because our previous study showed that droplets need ca. 800 μs to be homogeneously mixed after collision.[13] This is supported by the following estimation of the concentration ratio of the product, DNS-amide, to the reactant, DNS-Cl. Here, the estimation is performed based on the bulk properties. Elucidation of surface specificity requires more quantitative measurements. Assuming a pseudo-first-order reaction for the amidation of DNS-Cl, the concentration of DNS-amide, [DNS-amide], is given by

$$[\text{DNS-amide}] = [\text{DNS-Cl}]_0\,(1 - e^{-k[\text{IPA}]t}) \quad (1)$$

where [DNS-Cl], [IPA], k, and t represent the concentrations of DNS-Cl and IPA, the rate constant, and the reaction time, respectively. The suffix 0 indicates $t = 0$. At sufficiently small values of t, eq 1 is approximated as

$$[\text{DNS-amide}] \approx k[\text{DNS-Cl}]_0[\text{IPA}]t \quad (2)$$

And hence, the ratio of the product to the reactant is given by

$$[\text{DNS-amide}]/[\text{DNS-Cl}]_0 \approx k[\text{IPA}]t \quad (3)$$

Here, we use $k \approx 10\,\text{M}^{-1}\,\text{s}^{-1}$, based on the literature values of the hydrolysis rate of DNS-Cl ($2.4\,\text{M}^{-1}\,\text{s}^{-1}$) and the dansylation rates of amino acids and peptides ($2–50\,\text{M}^{-1}\,\text{s}^{-1}$).[19] The molar concentration of neat IPA is 11 M. Using these values, the concentration ratio of the product to the reactant is calculated to be 0.02–0.2 at $t = 2–20$ μs, indicating that the reaction proceeds during the early stages following collision. The observed kinetics include the mixing process as well, but the reaction kinetics is likely to dominate in the present study because this estimation reproduces the experimental values. In conclusion, the present method enables us to measure the rates of various rapid chemical reactions in solution. In the future, surface-specific reaction processes of solutions can also be elucidated by more quantitative measurements.

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