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APPLICATION NOTE

Study of Fluorescence Quenching Kinetics Using Stopped-Flow

AN_F39, 12 Dec, 2017, Stuart Thomson, Anna Gukasyan

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**Introduction**

The understanding of reaction kinetics is an important area of study across biology and chemistry. Through the study of reaction kinetics the reaction rate, reaction order and the underlying molecular mechanisms can be determined. A powerful method for determining the kinetics of a reaction is by monitoring the concentration of the reactants or products over time using absorption or fluorescence spectroscopy. For slow reactions, the reactants can simply be mixed by hand or using a magnetic stirrer. However, for fast reactions, where the lifetime of the reaction is comparable with the mixing time, more specialised techniques must be used such as stopped-flow.

Figure 1: FLS1000 Photoluminescence Spectrometer with Stopped-Flow Accessory

In stopped-flow the reactants are quickly forced into a cuvette where they rapidly mix and the change in absorbance or fluorescence intensity monitored over time. Edinburgh Instruments offers a stopped-flow accessory for both the FLS1000 and the FS5 Fluorescence Spectrometers, extending the capability of these systems for the measurement of rapid reaction kinetics. A model system for studying reaction kinetics is the fluorescence quenching of N-acetyltryptophanamide (NATA) by N-bromosuccinimide (NBS).[1] NATA is oxidised by NBS to form a non-fluorescent bromohydrin, as shown in Figure 2, and the kinetics of the reaction can therefore be investigated by monitoring the change in fluorescence intensity of NATA as a function of time.[1] By performing the reaction with an excess of NBS, the reaction exhibits pseudo-first order kinetics with a rate constant that is proportional to the concentration of NATA. At the concentrations used here this reaction is too fast to be monitored using manual mixing so it therefore serves as a useful verification test of the stopped-flow accessory.[1]

Figure 2: Oxidation of N-acetyl tryptophanamide (NATA) by N-bromosuccinimide (NBS).

**Methods and Materials**

N-acetyltryptophanamide and N-bromosuccinimide were dissolved in phosphate buffered saline (PBS) at pH 7.2. Three NATA solutions were prepared with concentrations of 5 µM, 10 µM and 20 µM. The NBS quencher was kept in excess with concentrations of 50 µM, 100 µM and 200 µM respectively. The absorption spectrum of NATA was recorded using the transmission detector of the FS5 SpectroFluorometer with an excitation bandwidth of 3 nm. Fluorescence measurements were performed using the FLS1000 Photoluminescence Spectrometer equipped with double excitation and emission monochromators, photomultiplier tube detector (PMT-980), a 450 W Xe lamp and the N-MDMM stopped-flow accessory.

The emission spectrum of NATA was recorded at an excitation wavelength of 280 nm using excitation and emission bandwidths of 3 nm and 1.5 nm respectively. For fluorescence kinetic measurements the NATA and NBS solutions were loaded into the A & B syringes of the N-MDMM stopped-flow accessory. Syringe C was filled with PBS buffer to serve as a reference. The TTL output trigger of the FLS1000 was used to initiate the N-MDMM injection and the total stop volume was set to 400 µl. The kinetic spectra were recorded using an excitation wavelength of 280 nm with a 5 nm bandpass, an emission wavelength of 360 nm with a 5 nm bandpass, and a temporal resolution of 0.2 ms.

**Results Discussion**

The absorption and emission spectra of NATA solution were recorded and shown in Figure 3. NATA has an absorption maximum at 280 nm and an emission maximum close to 360 nm and these were therefore chosen as the excitation and emission wavelengths for the subsequent fluorescence kinetic measurements.

Figure 3: Absorption (blue) and emission (red) spectra of NATA in PBS buffer.

A fluorescence kinetic of the quenching of 10 µM NATA by 100 µM NBS is shown in Figure 4 (red). The fluorescence quenching kinetic was analysed using the FLS1000 Fluorescence operating software. The data was fit well using a single monoexponential decay with a time constant of 28 ms.

The fluorescence kinetic was recorded with a high temporal

# Study of Fluorescence Quenching Kinetics Using Stopped Flow

In this application note the kinetics of NATA quenching by QBS are determined using a Photoluminescence Spectrometer equipped with a stopped-flow accessory.

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