FP APPLICATION NOTE 1-10

Acid Unfolding of Horse Cytochrome C Measured with a Fluorescence Stopped-Flow System

The fluorescence characteristics of tryptophan in proteins will vary depending on the structures surrounding the amino acid. The fluorescence of Cytochrome C is derived from the tryptophan in the residue position 59. The natural state of this tryptophan is so close to the Heme iron residue that the fluorescence is quenched by nonradiative energy transfer to the Heme iron. When Cytochrome C is denatured by an acid, the distance between the tryptophan and Heme iron changes and the fluorescence intensity grows. This application note demonstrates the measurement of the change in fluorescence intensity by the acid denaturation of Cytochrome C using the JASCO stopped-flow system.

Measurement/Analysis Systems

- FP-6500 Spectrofluorometer
- SFS-482 Stopped-Flow system (Cell length: 10 mm)
- [Stopped-Flow Measurement] program
- [Reaction Rate Calculation] program

Syringe configuration

S1: 10 mL, 0.5mg/mL Cytochrome C
S2: 10 mL, 0.1N sulfuric acid

Parameters:

Excitation bandwidth:	5 nm
Emission bandwidth:	5 nm
Response:	2 seconds
Sensitivity:	Manual
Excitation wavelength:	280 nm
Emission wavelength:	340 nm
Measurement range:	0-5000 milliseconds
Measurement interval:	5 milliseconds
No. of accumulations:	4
Flow time:	35 milliseconds
Flow volume:	S1 = 100 µl; S2 = 100 µl
Mixing ratio:	S1:S2 = 1:1

Experimental

Figure 1 illustrates the measured and calculated results of the Cytochrome C emission during the stopped-flow experiment.
The measured data shows an extreme change in the fluorescence intensity corresponding to the acid denaturation of Cytochrome C. The JASCO stopped-flow system enables data acquisition before the syringe movement is completed to ensure that the early stage of the reaction data before and after the flow time ends can be acquired.

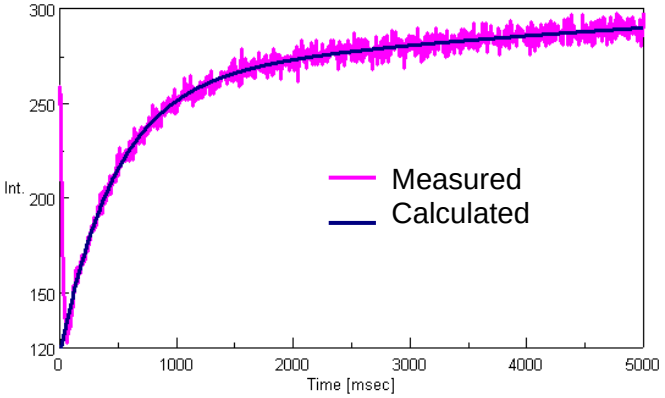

Figure 1: Measured and calculated results of Cytochrome C emission

The reaction rate was calculated with the [Reaction Rate Calculation] program. The calculated range was 35 to 5000 msec and a 2-step reaction mechanism was applied for the calculation. The calculated results show an excellent fit to the experimental data.

Calculation range:	35 to 5000 msec
Reaction rate formula:	Y(t) = -142.667 * exp(-t / 432.854) + -47.7112 * exp(-t / 3611.11)
Step 1 time constant:	432.854 msec
Step 1 rate constant:	0.00231025 $msec^{-1}$
Step 2 time constant:	3611.11 msec
Step 2 rate constant:	0.000276923 $msec^{-1}$

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