Application Note

FP-0007

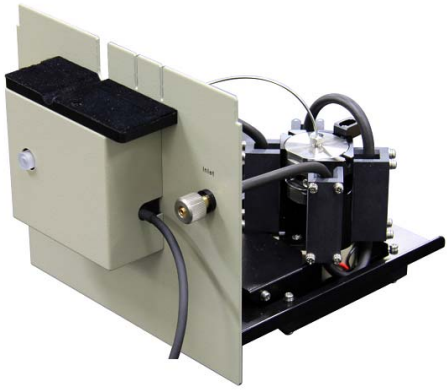
Acid Unfolding of Horse Cytochrome C Measured with a Fluorescence Stopped-Flow System

The fluorescence characteristics of the tryptophan residue in proteins will vary depending on the structures surrounding the residue. This characteristic of cytochrome C is derived from the tryptophan in the residue position 59. The natural state of this tryptophan residue 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 enlarges. This application note introduces the measurement example of the change in fluorescence intensity by the acid denaturation of Cytochrome C as measured by the JASCO stopped-flow measurement system.

Measurement/Analysis Systems

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

Stopped-Flow System



Cell Holder and Cell

Syringe configuration

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

Measurement Parameters

Ex bandwidth	5 nm
Em bandwidth	5 nm
Response	2 sec
Sensitivity	Manual
Measurement range	0 - 5000 msec
Measurement interval	5 msec
Ex wavelength	280 nm
Em wavelength	340 nm

No. of accumulations	4
Flow time	35 msec
Mixing ratio	S1:S2 = 1:1
Flow volume	S1: 100 µL S2: 100 µL
Start data acquisition	35ms before flow time ends

JASCO INTERNATIONAL CO., LTD
4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512

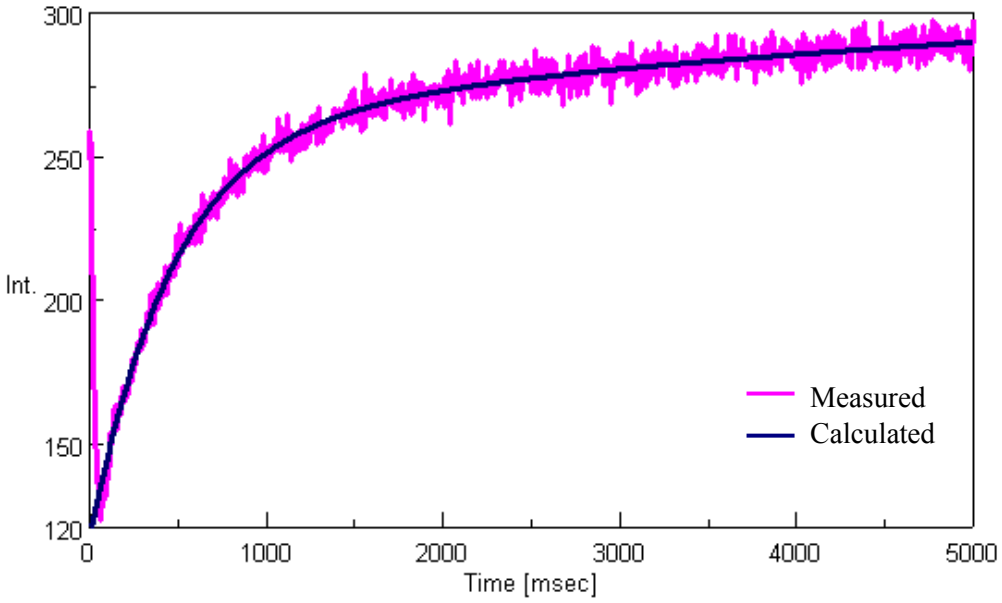


Figure 1: Measured and calculated results of Cytochrome C emission

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.
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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