Application Note
FP-0007

JASCO

Acid Unfolding of Cytochrome *c* Probed by Fluorescence Stopped-Flow

Introduction

Fluorescence spectroscopy can be used to monitor changes in the environment surrounding a protein's aromatic amino acid residues. Cytochrome *c* is a heme protein that has one tryptophan residue in close proximity to the heme group. In the protein's native state, the fluorescence is quenched by the non-radiative energy transfer from the tryptophan to the heme iron. When the protein unfolds under denaturation conditions, the distance between the tryptophan and heme increases, concomitantly increasing the fluorescence intensity. Coupling a stopped-flow system with a spectrofluorometer allows researchers to acquire information not only on the degree of structural changes to the protein, but on the unfolding kinetics as well.

SFS-852 Stopped-Flow Accessory

This application note demonstrates how stopped-flow fluorescence can probe the unfolding kinetics of cytochrome *c* denatured by sulfuric acid.

Keywords

FP-8500, Fluorescence, Stopped-flow, SFS-852 Stopped-flow system, Kinetics

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Experimental

Measurement Conditions			
Excitation Wavelength	280 nm	Emission Wavelength	340 nm
Excitation Bandwidth	5 nm	Emission Bandwidth	5 nm
Response Time	2 sec	Sensitivity	Manual
Measurement Interval	25 msec	Measurement Range	0-5000 msec
Accumulations	4	Flow Time	35 msec
Mixing Ratio	1:1	Flow Volume	S1, S2: 100 µL

10 mL of 0.5 mg/mL horse heart cytochrome *c* was placed in syringe 1 while 10 mL of 0.1 N sulfuric acid was placed in syringe 2. The data was acquired 35 milliseconds before the flow time ended.

Results

Figure 1 illustrates the measured (red) and calculated (blue) kinetic traces of cytochrome *c* reacting with sulfuric acid. The increase in fluorescence intensity indicates that the protein begins to unfold upon its interaction with sulfuric acid almost immediately and is completely unfolded around 2000 msec.

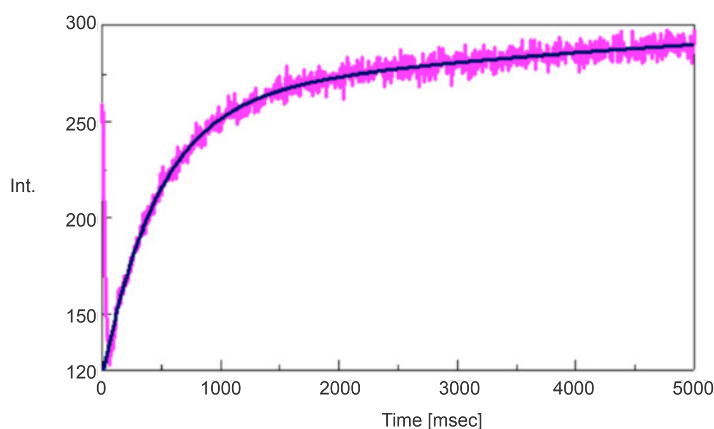

Figure 1. Measured (red) and calculated (blue) fluorescence emission spectrum of cytochrome c denatured with sulfuric acid.

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The reaction rate and time constants were calculated using the [Reaction Rate Calculation] program and the results are shown in Table 1. The calculated range was 35 to 5000 msec and a 2-step reaction mechanism was applied for the calculation. The calculated results are shown to fit the data.

Table 1. Kinetic parameters.

Rate Equation	$Y(t) = -570.486 \bullet e^{(-t/336.523)} + -217.93 \bullet e^{(-t/3039.55)}$
Step 1 Time Constant	336.523 msec
Step 1 Rate Constant	0.00297157 $msec^{-1}$
Step 2 Time Constant	3039.55 msec
Step 2 Rate Constant	0.000329 $msec^{-1}$

Conclusion

This application note demonstrates that the FP-8500 can be used to obtain thermal denaturation data for protein structure studies.

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