Refolding of Cytochrome *c*

Introduction

CD spectra provide information on the secondary structure of proteins and the environment of aromatic side chains. Therefore, CD measurement using a stopped-flow system is considered as one of the best methods for analyzing the unfolding and refolding of proteins. The existence of an intermediate between denaturated state and natural state during the refolding of proteins has been reported. The CD stopped-flow method is used for examining this refolding process. In this report the refolding process of cytochrome *c* (cyt c) measured using a SFS-492 stopped-flow system will be explained.

Keywords: Stopped-flow, Circular Dichroism, Refolding

Sample Preparation

Aqueous solution of Cytochrome *c* denaturated by guanidine hydrochloride (GuHCl) was diluted with 0.1 M acetic acid buffer solution (1:9). The refolding process was observed at 222 nm for the secondary structure and at 289 nm for the environment of the aromatic side chain.

Measurement conditions

Measurement system:	J-815 + SFS-492 stopped-flow system
Optical pathlength:	2 mm
Temperature:	Room temperature
Flow rate:	1.5 mL/sec

Each sample was prepared at an optimal concentration because CD intensities at 222 nm for the secondary structure and at 289 nm for aromatic side chains are quite different.

Wavelength	222 nm	289 nm
Data interval	5 msec	10 msec
Response	4 msec	8 msec
Bandwidth	4 nm	2 nm
Syringe 1	2 mg/mL cyt *c* /4.3 M GuHCl	10 mg/mL cyt *c* /4.3M GuHCl
Loading volume	30 µL	30 µL
Syringe 2	0.1 M acetic acid buffer solution (pH 6.3)	0.1 M acetic acid buffer solution (pH 6.3)
Loading volume	270 µL	270 µL
Accumulation	36 times	24 times

Results

The change in the CD value at 222 nm reflects fast refolding of the secondary structure within 200 msec (Fig. 1), but the change at 289 nm reflecting the environment of the aromatic side chain was slower (Fig. 2). This slower change appears in the latter step of the refolding process. These results indicate the brief existence of an intermediate state with a refolded secondary structure but with aromatic side chains remained unfolded.

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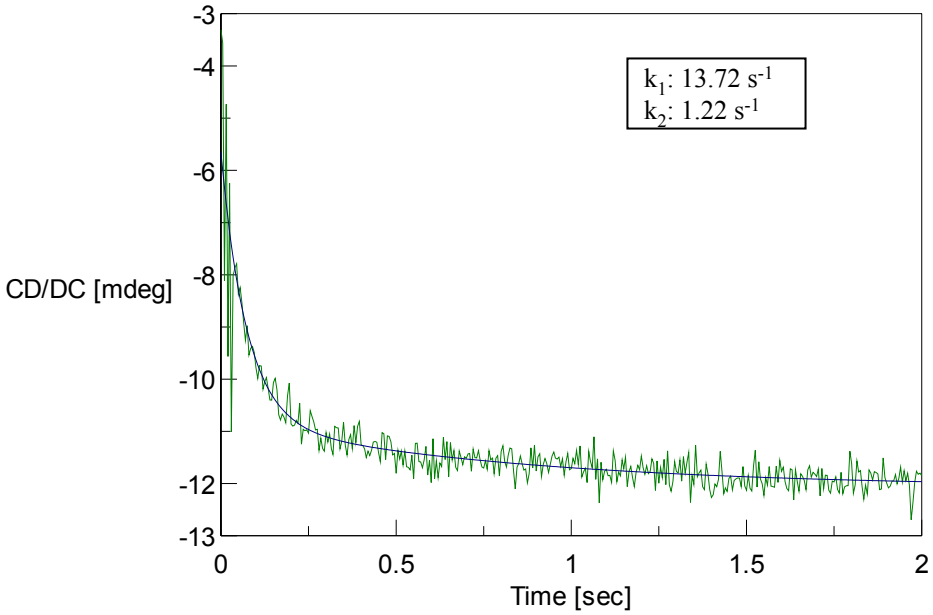

Fig. 1 Refolding measurement of cytochrome *c* (222 nm)

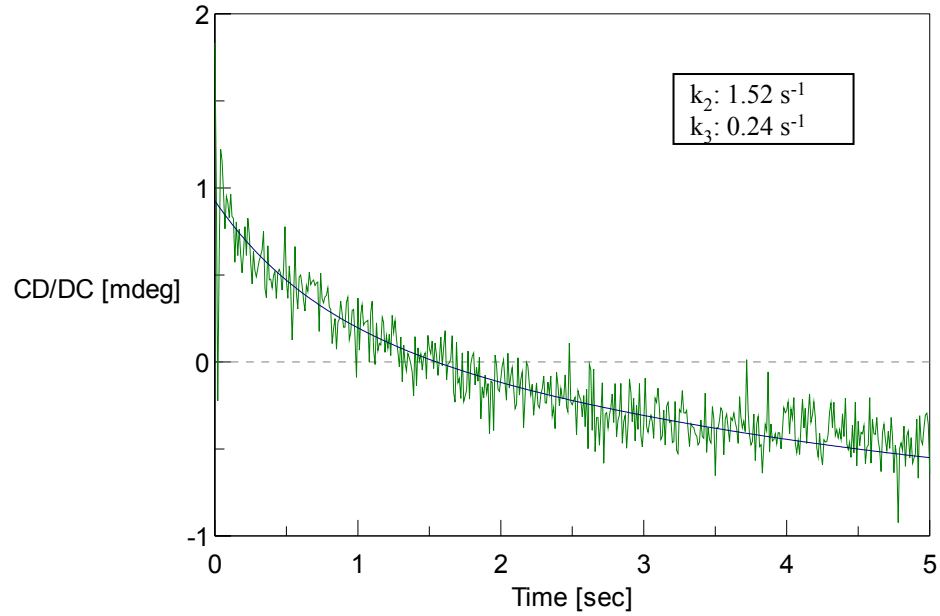

Fig. 2 Refolding measurement of cytochrome *c* (289 nm)

References

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