Submillisecond time-resolved small angle x-ray scattering from β-Lactoglobulin

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Introduction

Small angle x-ray scattering (SAXS) provides low-resolution structural information about macro-molecules in solution. It is therefore useful as a probe of large scale structural changes, such as those occurring during protein folding or unfolding. Using nanofabrication techniques, we have designed and constructed a rapid fluid mixer for synchrotron SAXS studies [1]. This flow cell allows us to modify the chemical environment of proteins on the submillisecond time scale, triggering folding or unfolding. Subsequent structural changes are monitored also on submillisecond time scales using the high intensity APS beams.

Methods and Materials

A schematic of the flow cell is shown in figure 1. Two crossed channels are etched through the thickness of a silicon wafer. The top and bottom surfaces of the wafer are sealed; fluid enters or exits at the end of each of the four channels. Solution A, a protein-containing fluid, is hydrodynamically focused into a micron-sized jet by solution B. Small ions/molecules rapidly diffuse out of the jet into the surrounding fluid. Diffusion of the protein is much slower as a result of its larger mass.

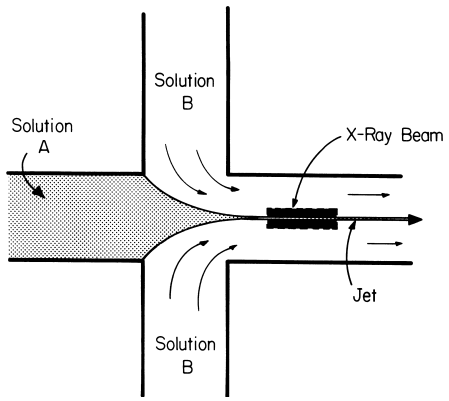

Figure 1: A cross sectional schematic of the rapid fluid mixer.

For these experiments we used the protein β-lactoglobulin (BLG), 162 amino acids, MW=18400. BLG can be extracted from cow's milk or expressed in *Pichia pastoris* [2]. Solution A contains (unfolded) BLG in the presence of 6M urea, a chemical denaturant. No urea is present in Solution B. X-ray exposures, taken at various positions along the outlet channel, record the structural changes as the protein folds in response to the rapid decrease in urea concentration.

Pink beam was employed in these experiments and is produced by reflecting the first harmonic of the APS undulator A (7.65 keV at a gap of 18 mm) off of a Si mirror. This 10 cm long mirror is horizontally deflecting, with a 0.15 degree fixed angle of incidence. The mirror cuts off the higher harmonics of the undulator and passes the first harmonic. Upstream of the sample, at a distance of 14 cm, the beam is collimated with a pair of crossed slits set to 10 microns in the vertical direction, so as to illuminate only the protein jet and 40 microns in the horizontal direction, so as to achieve the requisite time resolution of less than 100 microseconds per data point. The flux through the collimating pinhole was 2.5×10^{11} x-ray/second [3]. Scattered x-rays were detected using a home-built CCD detector[4], located 40 cm downstream of the sample.

Results

Figure 2 shows raw data from partially folded BLG at 2.5 mM, flowing in a nominally 5 micron wide jet. The plot is an angular integral over the 2d pattern from the CCD detector. The volume of protein seen by the x-ray beam is 80 picoliters. Data is acquired with exposure times of a few minutes.

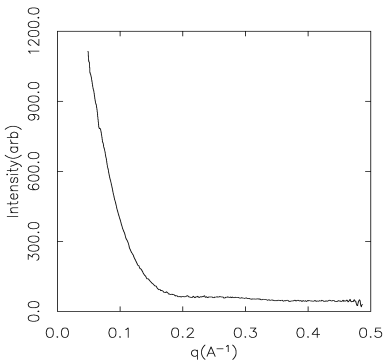

Figure 2: SAXS data from protein in mixing device.

Discussion

High intensity, pink beam, can be used in conjunction with a flowing sample to provide good-quality SAXS data. The short exposure times, in conjunction with small sample volumes, will allows us to obtain useful data from precious biological samples.

Acknowledgments

We acknowledge the valuable assistance of M. Novak and H. Gibson. This work was supported by the NSF under DMR-9701453 and by the DOE under DE-FG02-97ER62443.
This work was performed at IMM-CAT using the beamline ID-8. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38.

References

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