Flow Device for X-ray Spectroscopy Experiments

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Introduction

One of the challenges for synchrotron-based biological research, especially at the third-generation sources, is dealing with x-ray-induced damage to protein samples. It is believed that the damage is caused by the generation of free radicals. Measuring samples in a frozen solution state can reduce the damage. However, the extent of the reduction is not certain. Moreover, antifreeze is often added to the protein solution during low-temperature spectroscopy measurements to reduce the diffraction artifacts caused by the ice crystals. The antifreeze additive itself may accelerate the degradation of the samples [1].

Methods and Materials

One simple solution for maintaining a sample's integrity while it is being measured in the solution state is to flow the sample in the x-ray beam. With regard to implementing this solution, we have adapted our stop-flow system [2] for time-resolved x-ray absorption fine structure (XAFS) to a flow device. The stop-flow system uses servo motors to drive syringes for accurate and flexible sample delivery. With a new set of motors, we can control the flow rate from 0.1 to >100 μL/s. The stop-flow can also be cooled down to subzero temperatures. Thus, we can keep the sample cold during measurement. We used Kapton® tubing as the flow cell; the tube generally has an inside diameter of 1 mm, and its wall is as thin as 25 μm. The device is controlled by a PC base controller.

Figure 1 shows the flow device under testing at the Bio Collaborative Access Team (BioCAT) beamline (ID-18) at the APS. The flow device, seen in the center of the picture, has two motors mounted on top. The fluorescence signal is collected by using the multilayer analyzer array detector [3].

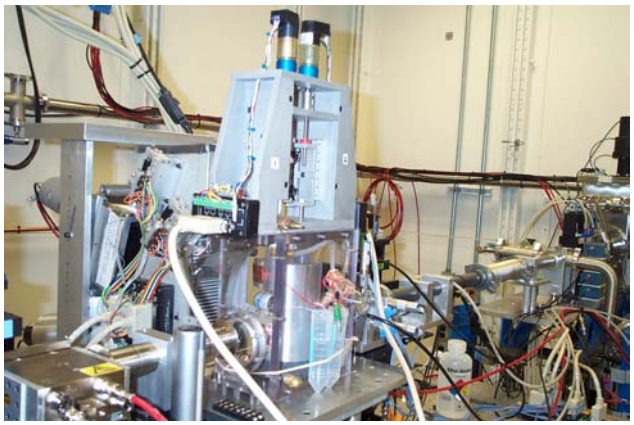

FIG. 1. Test of the flow device at beamline ID-18.

The flow device has been used by several groups taking x-ray spectroscopy measurements. With radiation-sensitive samples, an appropriate flow rate can be determined, and radiation-induced spectral changes will not occur for the measurements. Here we show the quality of data taken on a CuCl testing sample with the same setup.

Results

The tests were done on a 0.5 mM CuCl solution. No spectral variation occurred when the spectrum taken at a flow rate of 10 μL/s was compared with that taken at a flow rate of 1 μL/s. Therefore, we set the flow rate at 1 μL/s, with 250 μL total volume mounted in the device. The energy scan covered an 800-eV range with a time of 25 s per scan by using the continuous scan mode of the BioCAT. We collected 40 scans with the same sample. Thus the total data collection time was roughly 16 min.

The top graph in Fig. 2 shows a comparison of the sum of the first 10 scans (blue) with the sum of the last 10 scans (red). The spectra are essentially identical to each other, indicating that no radiation-induced changes occurred at the metal site. The step of the spectra at the end of the scan is due to the zinc impurity of the Kapton tubing. All 40 scans then summed together. XAFS χ data were derived from the spectrum by taking out the background, which is shown in the bottom graph in Fig. 2. Judging from the noise level of the spectrum, the data are good and analyzable.

Discussion

To summarize, we have added the continuous flow feature to our stop-flow device. The device is accurate and flexible in delivering solution samples to Kapton tubing for observation. When the device is combined with the multilayer detector, submillimolar protein solution samples can be measured in minutes while sample integrity is maintained.

Acknowledgments

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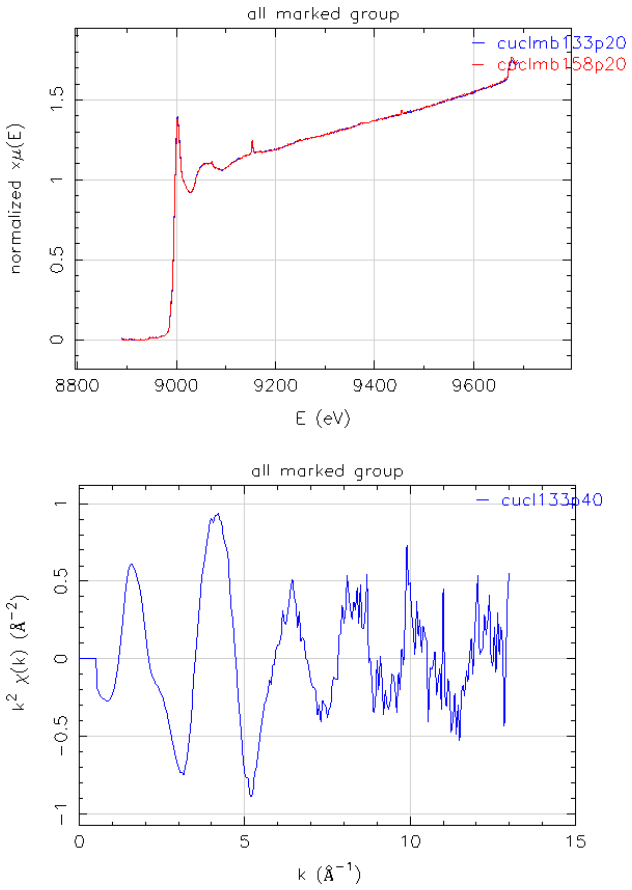

FIG. 2. Top shows a comparison of XAFS spectra for a 0.5 mM CuCl solution sample. Bottom shows the XAFS χ data derived from the total sum of the spectra.

References

[1] J. Penner-Hahn (private communication, 2001).

[2] K. Zhang et al. (to be submitted).

[3] K. Zhang, G. Rosenbaum, and G. Bunker, J. Synchrotron Rad. **5**, 1227-1234 (1998).