Cytochrome C and αTS Folding Probed by Submillisecond Continuous-Flow SAXS

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Protein folding dynamics is of great interest as being closely related to protein functions and the origin of many diseases. Many proteins collapse within the first hundred of microseconds thus requiring submillisecond-time resolution techniques to observe the effect. At the Advanced Photon Source we have applied a microfluidic continuous-flow mixer and a highly focused X-ray beam at the 18ID beamline in order to study protein folding by small-angle X-ray scattering (SAXS). This made possible to achieve time resolution of about 0.1 millisecond using final protein concentrations as low as 1 mg/ml. Refolding of guanidine-induced denatured state of cytochrome c studied by this technique in submillisecond and millisecond time range demonstrated progressive increase of compactness of the protein molecules indicated by the decrease in radius of gyration from 24 to 15 Å. The SAXS data from the α-subunit of tryptophan synthase demonstrated that the collapse of urea-denatured state of the protein occurred within the first 150 microseconds of dilution experiment. The measured radius of gyration of 33 Å was significantly smaller than that for the denatured state (43 Å). This work was supported by NIH grants RR08630 and GM23303 and NSF grant MCB0327504. Use of the Advanced Photon Source was supported by DOE under Contract No. W-31-109-Eng-38.

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