Kinetic refolding of α-lactalbumin studied by stopped-flow X-ray scattering

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To elucidate protein-folding mechanisms, it is important to detect and characterize a kinetic folding intermediate(s) accumulated transiently during folding. We have studied the folding process of proteins by stopped-flow solution X-ray scattering using synchrotron radiation to characterize molecular size and shape of the intermediates. We have previously shown that it is possible to improve dramatically the signal-to-noise ratio of time-resolved X-ray scattering measurement by use of a two-dimensional charge-coupled device (CCD)-based X-ray detector and that the data obtained by the detector are coincident with those obtained by a position-sensitive proportional counter after corrections for distortion of images, non-uniformity of sensitivity and contrast reduction of an X-ray image intensifier [1,2]. Using these techniques, here we measured a kinetic refolding reaction of α-lactalbumin (α-LA).

α-LA is a monomeric globular protein with molecular weight of 14,200, and is one of the best-studied proteins in protein folding studies. It has been known that α-LA forms a partially folded intermediate called a "molten globule state" at equilibrium and that it accumulates a kinetic folding intermediate resembling the molten globule state [3]. To characterize the molecular size and shape of the kinetic intermediate, we measured the kinetic refolding of α-LA by the stopped-flow X-ray scattering technique using the CCD-based X-ray detector. The X-ray scattering experiments were performed at BL-15A in Photon Factory, High Energy Accelerator Research Organization. Data were obtained at intervals of 10 ms, 100 ms, 500 ms, and 1 s. The same experiments were performed three times to check reproducibility of the measurement. The results show that a radius of gyration of the kinetic folding intermediate of α-LA accumulated within the dead time of the measurement (10 ms) is the same as that of the equilibrium molten globule state. Moreover, Kratky plots of the kinetic and equilibrium intermediates are coincident with each other (Figure 1). These data indicate that the kinetic folding intermediate of α-LA is equivalent with the equilibrium molten globule state in terms of the molecular size and shape.

The rate constant of refolding reaction subsequent to the formation of the molten globule-like intermediate is coincident with that measured by stopped-flow circular dichroism [4]. This indicates that formation of secondary structure and molecular compaction of α-LA take place simultaneously after the formation of the intermediate. Furthermore, in this process we observed the small decrease (~10%) in a forward scattering intensity ($I(0)$). The $I(0)$ value depends on the square of the difference in electron density between solute and solvent [5]. If the density of hydrated water molecules is different from that of bulk water [6], the decrease in $I(0)$ we observed can be due to dehydration of water molecules around hydrophobic side-chains of the intermediate during formation of specific side-chain packing.

References

[1] Y. Amemiya et al., Rev. Sci. Instrum. 66, 2290 (1995).
[2] M. Arai et al., *In* Old and New Views of Protein Folding (K. Kuwajima & M. Arai, eds.) pp. 31-40, Elsevier Science, Amsterdam, (1999).
[3] M. Arai & K. Kuwajima, Adv. Protein Chem. 53, 209 (2000).
[4] M. Ikeguchi et al., Protein Sci. 7, 1564 (1998).
[5] O. Glatter & O. Kratky, Small-Angle X-ray Scattering, Academic Press, London, (1982).
[6] D.I. Svergun et al., Proc. Natl. Acad. Sci. USA, 95, 2267 (1998).

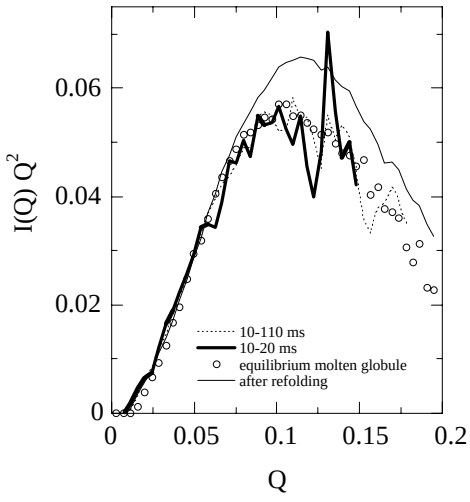

Figure 1: Kratky plots of α-lactalbumin in the molten globule state at equilibrium (pH 2, circles), after 10-20 ms of kinetic refolding (thick solid line), after 10-110 ms of refolding (thin dotted line) and in the native state after refolding (thin solid line).

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