Control of Protein Folding with Use of a Photo-trigger

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It is very useful to use photo-triggering methods to study biological events in proteins. We developed a new method to investigate the protein folding based on a pulsed laser light triggering method and a unique detection method. For triggering the folding reaction, a denaturant is photodissociated from a protein by a pulsed-laser. For monitoring the folding process, the time developments of the molecular volume change as well as the diffusion coefficient, which are difficult to detect by conventional spectroscopy, are monitored by the transient grating (TG) method. The side chain of the cysteine residue of apoplastocyanin (apoPC) was site-specifically modified with a 4,5-dimethoxy-2-nitrobenzyl derivative, where the CD and 2D NMR spectra showed that the modified apoPC was unfolded. The substituent was cleaved with a rate of about 400 ns by photoirradiation, which was monitored by the disappearance of the absorption band at 355 nm and the increase in the transient grating signal. After a sufficient time from the photocleavage reaction, the CD and NMR spectra showed that the native β-sheet structure was recovered. Protein folding dynamics was monitored in the time-domain with the transient grating method from a viewpoint of the molecular volume change and the diffusion coefficient, both of which reflect the global structural change including the protein–water interaction. The observed volume decrease of apoPC with a time scale of 270 μs is ascribed to the initial hydrophobic collapse. The increase in the diffusion coefficient (23 ms) is considered to indicate a change from an intermolecular to an intramolecular hydrogen bonding network.