

Formation of a compact structured ensemble early during ubiquitin folding investigated by stopped-flow CD, x-ray scattering and fluorescence

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As is β -lactoglobulin, we found ubiquitin, one of the smallest predominantly β -sheet proteins, also collapses first to a compact but not tightly packed state with excess helical structure.

Ubiquitin, used in the present experiments was Phe45Trp-mutated to have a tryptophan as a probe (abbreviated as Ub*).

The current work investigates the early stages of ubiquitin folding in viscous solvents and at low temperatures ($T = 4\text{ }^{\circ}\text{C}$ and $-20\text{ }^{\circ}\text{C}$). Ethylene glycol (EtGOH, 45 %) was added as an antifreeze.

The X-ray scattering measurements were taken at beamline 15A1. The X-ray wavelength was $\lambda = 1.50\text{ \AA}$ with either PSPC or CCD.

Results and discussion

The results for the non-equilibrium measurements at $-20\text{ }^{\circ}\text{C}$ are shown in Fig. 1. There are three phases present at both temperatures. At $-20\text{ }^{\circ}\text{C}$, a fast phase can be seen within the dead time (6 ms) of the apparatus in panels A and C. The fluorescence signal has minimal amplitude for the fast phase, but it does show the two slower phases clearly. The slowest phase can be seen in both fluorescence and circular dichroism data; it cannot be resolved in the X-ray scattering data due to its lower signal-to-noise-ratio. The $4\text{ }^{\circ}\text{C}$ X-ray data (not shown) shows a burst phase as well. Again the fluorescence is insensitive to this burst phase.

The CD value of the burst phase intermediate indicates greater-than-native helix content in the burst phase intermediate. This increase in helix goes hand in hand with a large reduction in the radius of gyration of the protein, as can be seen in Fig. 1 where R_g of the protein decreases by 6 \AA in $< 6\text{ ms}$. At $4\text{ }^{\circ}\text{C}$ the CD signal also shows a rapid decrease (data not shown) but no phases after the

initial rapid decrease are resolved. The R_g at this temperature decreases by 9 \AA within 6 ms.

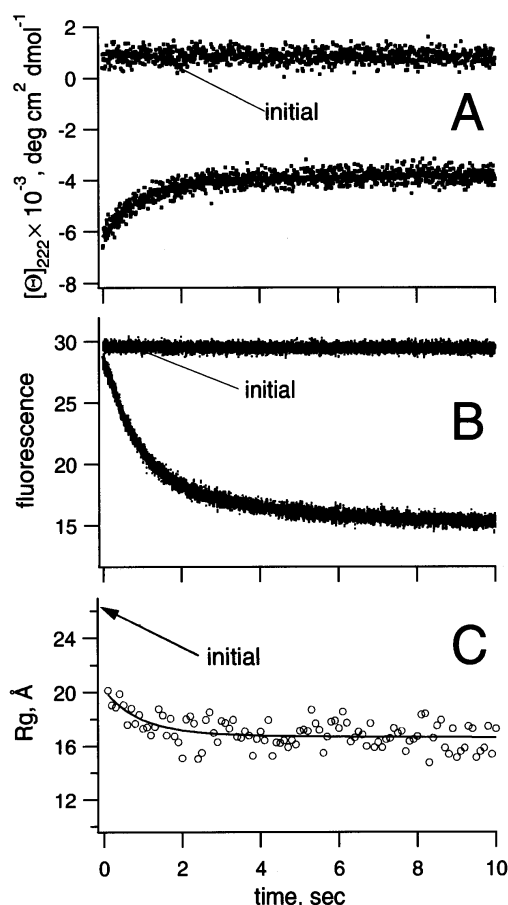


Figure 1: Ub*, -20° refolding in 45% EtGOH. The refolding was followed by CD at 222 nm (A), 295 nm excited fluorescence integrated above 335 nm (B), and R_g (C). The three data sets were fit to a double exponential decay as described in the text. The fit is shown as a solid line through the points.

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