OXIDATIVE FOLDING PROCESS OF AMARANTHUS α-AMYLASE INHIBITOR

Maša Čemažar,[1] Sotir Zakhariev,[1] Jakob J. Lopez,[2] Oliviero Carugo,[1,3] Jonathan A. Jones,[4,5] Peter J. Hore[2,5] and Sandor Pongor[1]

[1] *International Centre for Genetic Engineering and Biotechnology, Trieste, Italy;*
[2] *Physical and Theoretical Chemistry Laboratory, Oxford University, UK;*
[3] *Department of General Chemistry, University of Pavia, Italy;*
[4] *Clarendon Laboratory, Oxford University, UK;*
[5] *Oxford Centre for Molecular Sciences, Oxford University , UK.*

Oxidative protein folding comprises two main processes: i) formation of native disulfide bridges, sometimes via reshuffling and non-native intermediates, and ii) recovery of the native tertiary structure (conformational folding). We have characterised in detail the oxidative folding of a small cysteine knot protein, Amaranthus alpha-amylase inhibitor [1,2] (AAI, 32 amino acids, 3 disulfide bridges) with RP-HPLC, electron spray ionization mass spectroscopy (ESI-MS), 1H NMR and photo-chemically induced dynamic nuclear polarization (photo-CIDNP).

Disulfide intermediates of the folding process were isolated using the acid-trapping technique and RP-HPLC and their disulfide pairings were determined by combining enzymatic digestion and LC-MS[3]. Interestingly, the five most abundant intermediates were fully oxidized, three-disulfide species. Moreover, it was also revealed that three of the five fully oxidised species involved a rare structural element, a vicinal disulfide bridge (Cys17-Cys18).

The tertiary folds of the species present along the folding process have been elucidated using equilibrium and time-resolved NMR and photo-CIDNP[4]. The latter is an NMR method, which probes the surface accessibility of the aromatic side chains (Tyr-21, Tyr-27, Tyr-28, Trp-5). The equilibrium spectra of purified species were compared with time-resolved spectra taken at various time intervals along the folding pathway. The findings were consistent with RP-HPLC and ESI-MS time-resolved measurements.

The molecular dimensions of the native protein and the main folding intermediate (MFI) species were compared by performing NMR pulsed field gradient diffusion measurements. Even though native and MFI possess different disulfide pairings, their sizes are indistinguishable within the experimental error. Additional results from 2D NMR experiments led to the conclusion that the MFI state of AAI is compact and well-ordered, with one major and at least one minor conformation.

The results give a comprehensive picture of oxidative folding as a contemporary search for the correct fold and the correct disulfide pairing. MFI may play a role similar to that of the molten globule state of larger proteins by constraining the peptide chain to a smaller number of conformations that can be rapidly funnelled towards the native state.

References

[1] Chagolla-Lopez, A., Blanco-Labra, A., Patthy, A., Sanchez, R. and Pongor, S. (1994) J Biol Chem 269, 23675.

[2] Lu, S. and Pongor, S. et al (1999) J Biol Chem 274, 20473.

[3] Cemazar, M. et al, in preparation (2002).

[4] Maeda, K., Lyon, C. E., Lopez, J. J., Cemazar, M., Dobson, C. M. and Hore, P. J. (2000) J Biomol NMR 16, 235.