ATOM BY ATOM ANALYSIS OF THE FOLDING OF GPW

L. Sborgi, E. De Alba, V. Munoz

Centro de Investigaciones Biologicas, Consejo Superior de Investigationes Cientificas (CSIC),
Madrid, 28040, Spain

GPW is a midsize single gene product protein domain from bacteriophage λ with a 3D structure consisting of a two stranded, antiparallel β-sheet packed against two α-helices to form a single hydrophobic core[1]. The size and α+β topology of this domain suggest slow two-state-like folding over a large barrier. However, nanosecond temperature-jump experiments show that this protein folds in the microsecond time scale, expanding the realm of ultrafast folding. Both, thermodynamic and kinetic experiments indicate that the maximal folding barrier height for GPW is about 1RT[2], making this protein an ideal candidate for the atom-by-atom analysis of protein folding using high resolution NMR[3]. Here we report preliminary multidimensional heteronuclear NMR experiments on GPW toward that direction. Changes in ^{15}N, ^{13}C and ^{1}H chemical shifts with temperature have been monitored for all the signals in the low temperature range of the equilibrium thermal unfolding of GPW. From these experiments we see that the ensemble average chemical shifts (the protein is in the fast chemical exchange regime) can be assigned and followed for the largest part of the signals in GPW. Our results in the low temperature range allow us to establish the baselines for the atomic environments of the native structure and already hint the presence of structuralconformational heterogeneity during the early stages of thermal unfolding. The atom-by-atom analysis of GPW will provide important insights into the degree of the network of noncovalent interactions stabilizing the protein and its folding mechanism.

[1] Maxwell, K.L.; Yee, A.A.; Booth, V.; Arrowsmith, C.H.; Gold, M.; Davidson, A.R., *J.Mol.Biol.*, **2001**, *308*, 9-14.
[2] Fung, A.; Li, P.; Godoy-Ruiz, R.; Sanchez-Ruiz, J.M.; Munoz, V., *J.Am.Chem.Soc.*, **2008**, *130*, 7489-7495.
[3] Sadqi, M.; Fushman, D.; Munoz V., *Nature*, **2006**, *442*, 317-321.

This work was supported by Marie Curie Excellence Award MEXT-CT-2006-042334 to V.M.