MODEL STUDY ON THE FOLDING OF A NASCENT POLYPEPTIDE CHAIN USING SOLUTION NMR

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Folding of nascent polypeptide chains has long been of keen interest for biochemists and structural biologists, both for the biological interest and in connection with the medical problem of misfolded-protein diseases, such as transmissible spongiform encephalopathy (TSE), Alzheimer's disease, Parkinson's disease, various amyloidoses and possibly certain types of cancer. NMR studies of nascent polypeptide chains attached to the ribosome are typically limited by the large size of the complex. In this model study we substitute the ribosome with the GB1 domain as a solubility and stabilization tag for fragments of the protein NsP1, in order to obtain structural data characterizing fragments of the polypeptide which occur transiently during the biosynthesis. The protein NsP1 contains 106 residues, which form a six-stranded beta-barrel and one alpha-helix in a globular domain of 12.8 kDa. We designed six fragments of NsP1 to represent the polypeptide chain at various stages of the biosynthesis. Thus far we expressed and characterized the 3D structure of two short constructs, i.e., NsP1-D26/GB1/His and NsP1-T51/GB1/His, which include 15 and 40 amino acids of NsP1, respectively. Secondary structures were identified based on the differences of the $^{13}C_\alpha$ and $^{13}C_\beta$ chemical shifts from the sequence-corrected random-coil values, and the polypeptide backbone dynamics was assessed by measuring $^{15}N\{^1H\}$-NOEs. In NsP1-D26/GB1/His the NsP1 fragment appears to be in an extended disordered conformation. In NsP1-T51/GB1/His the NsP1 fragment includes a segment that shows pronounced $\alpha$-helix propensity (residues 37-48), which corresponds to a well-defined $\alpha$-helix in the full-length NsP1 globular domain. This polypeptide segment also has reduced flexibility (positive $^{15}N\{^1H\}$-NOEs). Structural studies of the NsP1-S101/GB1/His protein are underway.