

STUDY OF H/D EXCHANGE KINETICS OF LINEAR AND CYCLIZED PROTEIN USING MASS SPECTROMETRY

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Electrospray ionization mass spectrometry (ESI-MS) is a complementary method to NMR spectroscopy for studying protein folding/unfolding.¹ In the present work, the N-terminal domain of *E. coli* DnaB helicase (DnaB-N) was used as a model system to assess the stabilization against unfolding gained by covalent cyclization of proteins. Cyclization was achieved via formation of an amide bond between the N- and C-termini using a split mini-intein.² Cyclized proteins were prepared that contained linking peptides of varying lengths (3-9 amino acids) to join the N- and C-termini. The corresponding linear proteins were also prepared. Hydrogen-deuterium exchange experiments were carried out using a Waters Q-TOF2 ESI mass spectrometer. As proteins unfold (or fold), amide protons exchange for deuterons, resulting in an increase in the mass of a protein. The data can be processed to determine the extent and rate of unfolding (folding). The aims of this study were to compare the rates of unfolding of linear and cyclized DnaB-N (helicase) proteins, and to compare the effects of varying linker lengths on unfolding. These experiments showed that DnaB-N exhibits rare EX1 unfolding kinetics.³ Furthermore, the cyclized proteins were more stable than their linear counterparts.

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