Stability and Folding of a Mutant Ribose Binding Protein of Escherichia coli

Joon-Sik Kim* and Hyoungman Kim

Department of Biological Sciences, KAIST, 373-1 Kusong-dong, Yusong-gu, Taejon 305-701

A mature mutant ribose binding protein (RBP) of Escherichia coli was obtained by site-directed mutagenesis, replacing Thr-3 in the N-domain of wild-type mature RBP (WT-mRBP) with a Trp residue (N-Trp-mRBP). The equilibrium unfolding properties and the refolding kinetics of this protein were monitored by fluorescence and circular dichroism (CD). The stability of N-Trp-mRBP appears to be the same as that of C-Trp-mRBP, another mutant obtained by replacing Phe-187 with a Trp, and lower than that of WT-mRBP. The overall refolding rate of N-Trp-mRBP was much smaller than that of C-Trp-mRBP which, in turn, is only marginally smaller than that of WT-mRBP. For the case of WT-mRBP, the rate constant obtained Tyr fluorescence was identical to the value obtained by CD. But for the case of C-Trp-mRBP, the rate constant from CD was smaller than the value from the Trp-fluorescence and this difference in the rate constants was much greater with the N-Trp-mRBP.