SI-4

^{15}N NMR Relaxation Studies of Ketosteroid Isomerase

Sunggoo Yun, Do Soo Jang, KwanYong Choi, and Hee Cheon Lee

Department of Chemistry & Division of Molecular and Life Sciences
Pohang Universtity of Science and Technology, Pohang, 790-784, Korea

ABSTRACT

The backbone dynamics of Δ^5-3-ketosteroid isomerase (KSI) from *Pseudomonas testosteroni* has been studied in free enzyme and its complex with a steroid ligand, 19-nortestosterone hemisuccinate (19-NTHS), by ^{15}N relaxation measurements. The relaxation data were analyzed using the model-free formalism to extract the model-free parameters (S_2, τ_e, and R_{ex}). The results show that a large number of the residues exhibit reduced order parameters (S^2) upon ligand binding. In particular, the strands B3, B5 and B6, which have most of the residues involved in the dimer interaction, have the reduced order parameters in the steroid-bound KSI, indicating the increased high-frequency (pico- to nanosecond) motions in the interface region of this homodimeric enzyme. The backbone dynamics of KSI has also been studied in the presence and absence of 5 % trifluoroethanol (TFE) to explain the increased enzyme stability by TFE in the equilibrium unfolding process by urea. The presence of 5% TFE causes little change or slight increase in the order parameters for a number of residues, which are located mainly in the dimer interface region. However, the majority of the residues exhibit reduced order parameters in the presence of 5 % TFE, indicating that the entropy can be an important factor for the enzyme stability, and the increase in entropy by TFE is partially responsible for the increased stability of KSI.