Residue-specific thermodynamic and kinetic NMR study of the two-state protein transition provides insight into the transition state structure

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論 文 名　：Residue-specific thermodynamic and kinetic NMR study of the two-state protein transition provides insight into the transition state structure
（NMR を用いた蛋白質のアミノ酸レベルでの速度論・熱力学的解析によって遷移状態の構造を明らかにする）

区　　分　：甲

論 文 内 容 の 要 旨

EXSY (exchange spectroscopy) NMR provides the residue-specific equilibrium constants, *K*, and residue-specific kinetic rate constants, *k*, of a polypeptide chain in a two-state exchange in the slow exchange regime. A linear free energy relationship (LFER) discovered in a log *k* versus log *K* plot is a physicochemical basis for smooth folding and conformational changes of protein molecules. For accurate determination of the thermodynamic and kinetic parameters, the measurement bias arising from state-specific differences in the R_1 and R_2 relaxation rates of ^{1}H and ^{15}N nuclei in ^{1}H-^{15}N HSQC and EXSY experiments must be minimized. Here, we showed that the time-zero HSQC acquisition scheme (HSQC0) is very effective for this purpose, in combination with a special analytical method (Π analysis) for EXSY. As an example, we applied the HSQC0+Π method to the two-state exchange of nukacin ISK-1 in an aqueous solution. Nukacin ISK-1 is a 27-residue lantibiotic peptide containing three mono-sulfide linkages. The resultant bias-free residue-based LFER provided valuable insights into the transition state of the topological interconversion of nukacin ISK-1. We found that two amino acid residues were exceptions in the residue-based LFER relationship. We inferred that the two residues could adopt special conformations in the transition state, to allow the threading of some side chains through a ring structure formed by one of the three mono-sulfide linkages. In this context, the two residues are a useful target for the manipulation of the physicochemical properties and biological activities of nukacin ISK-1.

The SH3 domain from spectrin (spcSH3) is in an equilibrium between the native state F and the denatured state U in an acidic pH solution. The spcSH3 is a rare protein in which folding/refolding with large conformational changes can be directly monitored by NMR due to the slow exchange rates between the two states. We applied the HSQC0 and EXSY-Π method to measure the accurate residue-specific equilibrium constants, *K*, and rate constants, *k*, of the spcSH3. We then determined multiprobe ϕ values (ϕ_{multi}), which are a special type of ϕ values (per-residue fractions of the state F in the transition state) calculated based on the changes in the residue-specific equilibrium constants and rate constants caused by a single amino acid mutation. A set of the ϕ_{multi} values of a protein molecule could reveal the cooperativity with residue-level resolution in the transition state of protein structural changes.