Analysis of conformational space sampled by domain reorientation in linear diubiquitin by paramagnetic NMR

（常磁性NMRによる直鎖ジユビキチンのコンフォメーション空間の解析）

HOU XUENI

Ubiquitination is an important post-translational modification, involving the formation of an isopeptide bond between C-terminus of ubiquitin and ε-amine group of lysine in substrates, which plays a variety of regulatory roles in cellular processes. Ubiquitin is a highly conserved 76 amino acid polypeptide of ~8500Da, adopting a compact β-grasp fold with a highly flexible C-terminal tail. More importantly, it could be used as an acceptor for generating polyubiquitin chains conjugated by an isopeptide bond between C-terminus of Gly76 of one ubiquitin and one of ε-amine group of Lys6, Lys11, Lys27, Lsy29, Lys33, Lys48, or Lys63 of another ubiquitin, significantly improving the accuracy, efficiency, and complexity of recognition with diverse partners. In addition, α-amine group of Met1 could be used for ubiquitin attachment in tandem to generate Met1-linked polyubiquitin chains, also known as linear polyubiquitin chains, which are mainly involved in the regulation of immune signaling.

It is critical to characterize the conformational space of polyubiquitin chains in solution to understand their psychological behaviors. Paramagnetic NMR is a powerful analytical technique to study the dynamics of polyubiquitin chains, as it could provide long-range restraints (< 60 Å). In this study, paramagnetic restraints were acquired to derive a more comprehensive view of the conformational space of unanchored linear diubiquitin, which is the minimal structural unit of linear polyubiquitin chains.

Chapter I General introduction

Chapter I outlined the background information about the ubiquitin codes, including the cellular functions and complexity of their characterized conformations. It also introduced the theoretical basis of paramagnetism induced through paramagnetic ions, as well as the principles of ensemble reconstructions based on acquired paramagnetic restraints.

Chapter II Paramagnetic NMR-based structural analysis of linear diubiquitin in solution

Chapter II reported a study to determine the conformational space sampled by the relative position and orientation of two ubiquitin units in a linear diubiquitin molecule, using two

different ensemble averaging approaches based on a large number of paramagnetic restraints, especially pseudocontact shifts (PCSs) and residual dipolar couplings (RDCs), induced through a paramagnetic ion positioned at different positions (in both units). The results suggested that although the linear diubiquitin chain exhibits no extensive Ub/Ub interactions and a highly dynamic behavior, its conformational sampling is not completely random, while some preferred regions are more frequently visited. In addition, the intrinsic properties of paramagnetic restraints acquired from derivatives with different paramagnetic centers were illustrated. The results indicated that even the spin labels introduced do not directly interfere with the possible conformations, the inevitable deficiency in the information content of acquired paramagnetic data should be carefully assessed before characterizing the conformational diversity, as it might increase or decrease the tendency towards specific arrangements of two domains. The calculations also confirmed that PCS and RDC, which could be measured through the same paramagnetic ions with non-vanishing magnetic anisotropy susceptibility, are highly complementary.

Chapter III Probing conformational fluctuation of linear diubiquitin in solution

Chapter III described the possible factors, hydrophobic interactions, electrostatic interactions, and intrinsic backbone flexibility of linkage comprising of several amino acid residues, function in the formation of determined major states in Chapter II. The results suggested that perturbation of conformational space could be dominantly achieved by modifications of linkage. Electrostatic interactions between some charged amino acid residues could stabilize the compact conformation of linear diubiquitin. In the combination of kinetic measurements using biolayer interferometry (BLI), structural insights into the relationship between the conformational space and the target binding mode of linear diubiquitin are somewhat clear. In addition, the possible effect of the inevitable uncertainty of magnetic anisotropy susceptibility on ensemble reconstruction was carefully assessed by two approaches.

Chapter IV Specific recognition of linear diubiquitin by the Npl4 zinc finger (NZF) domain of HOIL-1L

Chapter IV illustrated the specific recognition of linear diubiquitin by Npl4 zinc finger domain of HOIL-1L by NMR titration measurements and paramagnetic NMR. The results suggested that even sparse PCS restraints could provide precious structural information on the possible ligand-driven conformational change in solution, distinct from the complex structure in a static state determined by crystallography.

Chapter V Thesis conclusions

Chapter V provided a general conclusion on the dynamic behaviors of unanchored linear diubiquitin, as well as the potentials and shortcomings of paramagnetic restraints induced through paramagnetic ions on the representation of conformational heterogeneity in linear diubiquitin system.