Title：Conformational Dynamics of Mitochondrial Import Proteins: A View from Molecular Dynamics Simulations on Experimental Data
(ミトコンドリアタンパク質輸送体サブユニットの運動と構造： 分子動力学シミュレーションで見る実験データ)
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Summary of Thesis

Most of the mitochondrial proteins are imported into mitochondria from the cytosol. This import process is governed by the coordinated actions of two translocases, translocase of the outer membrane (TOM) and the translocase of the inner membrane (TIM). First, we studied Tom20, a subunit of TOM complex which binds to a targeting signal (presequence) present on the proteins being transferred. Due to the low affinity between Tom20 and presequence, disulfide-bond tethering between Tom20 and presequence was required to obtain the X-ray structure of the Tom20-presequence complex. NMR and X-ray studies of Tom20-presequence complexes suggested the existence of a dynamic-equilibrium between Tom20 and presequence, which was believed to be the basis of their recognition. We performed molecular dynamics (MD) simulations on tethered Tom20-presequence complexes which revealed minimal effects of the disulfide-bond tethering on the protein dynamics suggesting that this technique is useful to study the dynamics of weakly binding complexes. In addition, a new X-ray structure of Tom20-presequence complex was also obtained using a relatively new technique called crystal contact-free space (CCFS). This is a special protein design method to explore dynamics where, in principle, the mobile region of the protein has very minimal crystal contacts. We performed crystal MD simulations of Tom20-presequence CCFS complexes which showed that CCFS could reproduce dynamics observed in solution. We also studied, Tim21, a subunit of the TIM23 complex. X-ray, NMR and CCFS X-ray structures showed a highly flexible loop segment, located in the vicinity of the binding site of Tim23. MD simulations starting from these three structures could not explain the conformational differences observed in experiments. To avoid the experimental bias, the loop conformation was modeled computationally and MD simulations were performed starting from ten best models. These simulations revealed a complex conformational ensemble with the CCFS structure more feasible as compared to the X-ray and NMR structures. In conclusion, MD simulations showed that these experimental designs, based on X-ray crystallography, to study weakly binding complexes and conformational dynamics of intrinsically flexible regions reproduce solution dynamics and could therefore be helpful to study other biological systems.