NMR Approach for Investigation of Large Molecule Systems
Ultra High Field MRI for Molecular Brain Imaging

Hideo Akutsu, PhD (Osaka University)

The size limit is a serious problem for solution NMR. The size limit is caused by the signal broadening due to rapid transverse relaxation in a slowly tumbling molecule and by the signal overlapping due to the huge number of resonating nuclear spins in a protein. The former has been improved by means of deuteration of the protein and innovative pulse techniques. Development of TROSY spectroscopy has contributed to a revolutionary improvement of the resolution of $^{1}H-^{15}N$ and $^{1}H-^{13}C$(aromatic) correlation spectra. The combination of deuteration and TROSY raised the molecular mass limit to more than 50 kDa.

F_OF_1-ATP synthase is a multisubunit enzyme that catalyzes ATP synthesis in oxidative phosphorylation and photophosphorylation. This enzyme consists of two components, F_O and F_1. The simplest F_1 (F_1-ATPase) comprises five kinds of subunits with a stoichiometry of a_3b_3gde. The molecular mass is about 360 kDa. In the crystal structure of F_1 from bovine heart mitochondria (MF_1), the three catalytic sites are not equivalent. The *b* subunit in F_1 takes on the closed form in the presence of a bound nucleotide, while it takes on the open form in its absence. We have applied segmental isotope-labeling by intein splicing reaction to the *b* subunit of F_OF_1-ATP synthase in this work, and have succeeded in obtaining the detailed information on the conformational change of the *b* subunit monomer (1).

Solid-state NMR is a promising method for investigations of membrane proteins and supramolecular systems because there is no size limit. We have been developing multidimensional solid-state NMR under MASS for the structural analysis of uniformly and specifically isotope-labeled samples (2-4). Newly developed methods were applied to H^+-ATP synthase g subunit and Mastoparan X bound to the lipid membrane. Mastopran X is a wasp venom and known to activate a G-protein. Uniformly and specifically labeled Mastoparans X were bound to DPPC-DPPG bilayer membranes under a hydrated condition. Its structure was successfully determined.

It can be concluded that the combination of solution and solid-state NMR is a powerful approach to investigate a large molecule systems.

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