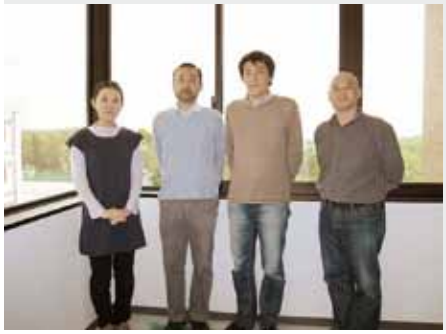
Solid State NMR for Molecular Science

Department of Materials Molecular Science
Division of Molecular Functions



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We are working on methodology and hardware developments of solid state NMR and structural biology and materials molecular science. In the following, we show studies of peripheral membrane proteins and inorganic compounds based on NMR.

1. NMR and Native-PAGE Analyses of the Phospholipase C-δ1 Pleckstrin Homology Domain

Phospholipase C (PLC) binds to phosphatidylinositol 4,5-bisphosphate (PIP_2) in the cell membrane through the pleckstrin homology (PH) domain, and hydrolyzes PIP_2 to produce two second messengers, diacylglycerol and inositol 1,4,5-triphosphate (IP_3), by the catalytic domain. The PLC-δ1 PH domain has a characteristic short α-helix (α2) from residues 82-87 (Figure 1). Solid-state ^{13}C NMR studies of the PLC-δ1 PH domain suggested that the α2-helix non-specifically interacts with the hydrophobic layer of the membrane due to the membrane localization of the protein.[1)]

In this study, the contributions of the α2-helix toward the IP_3 binding activity and thermal stability of the PLC-δ1 PH domain were therefore investigated by using NMR and Native-PAGE methods.

Native-PAGE analyses indicated that IP_3 binding to the PLC-δ1 PH domain results in a drastic migration shift on the gel and in an increased thermal stability. In addition, we found that disruption of the α-helical conformation by replacement of Lys-86 with proline resulted in reduced affinity for IP_3 and in thermal destabilization of the IP_3-binding state. Although the mutant protein with replacement of Lys-86 with alanine showed a slight reduction in thermal stability, the IP_3-binding affinity was similar to that of the wild-type protein. Replacement of Phe-87 with alanine, but not with tyrosine, also resulted in reduced affinity for IP_3 and in thermal instability. These results indicated that the helical conformation of the α2-helix and the phenyl ring of Phe-87 play important roles in the IP_3-binding activity and thermal stability of the PLC-δ1 PH domain.[2)] The ^{1}H-^{15}N HSQC NMR study of the selectively [α-^{15}N]Lys-labeled PLC-δ1 PH domain indicated that

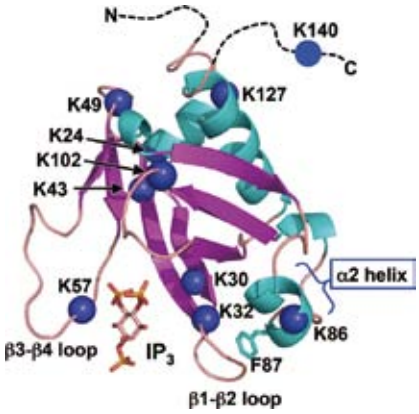

Figure 1. Crystal structure of the PLC-δ1 PH domain complexed with IP_3 (PDB ID, 1MAI).

IP_3 binding induces chemical shift displacement of all lysine signals (Figure 2). Interestingly, among those signals, only line width of α-^{15}N signal from Lys-86 located on the α2-helix markedly changed due to IP_3 binding (Figure 2, inset): in the ligand-free form, the α-^{15}N signal line width of Lys-86 was about two-fold broader than those of the other lysine signals in the one-dimensional ^{15}N projection of the ^{1}H-^{15}N HSQC NMR spectrum, and the addition of IP_3 resulted in line narrowing, and that the broad line width of the [α-^{15}N]Lys-86 signal converts to a narrow line width with addition of IP_3 (Figure 2). These findings provide evidence that the conformation and/or dynamics of the α2-helix couple with the ligand-binding activity of the PLC-δ1 PH domain. These findings suggested that the conformational changes of the α2-helix induced by membrane binding result in conversion of the stereospecific ligand-binding site to a weak-affinity site and in protein instability. Based on these results, we propose a new affinity regulation mechanism in which the ligand stereospecificity of the PLC-δ1 PH domain is significantly reduced due to protein structural changes by the membrane binding, and non-specific membrane binding or insertion is therefore important for stable anchoring to the lipid membrane. These findings also suggest that the ligand stereospecificity of the protein mainly contributes to searching the membrane contain-

ing PIP_2 and that stable anchoring to the lipid membrane is mainly achieved by non-specific membrane binding or insertion rather than PIP_2 binding. This mechanism also explains the lower affinity of the protein to lipid bilayers than IP_3, which is an essential property for feedback control of catalytic reaction of PLC-δ1 with respect to PIP_2.

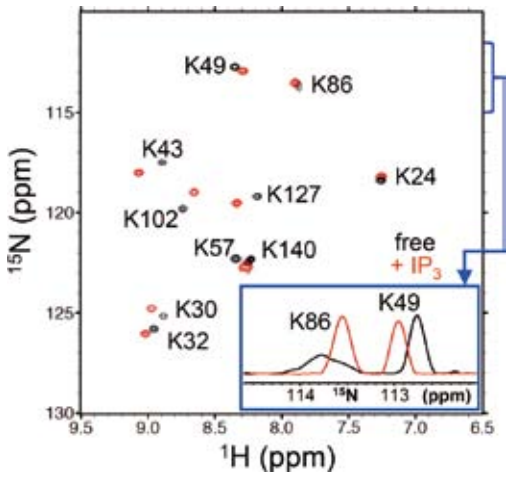

Figure 2. Solution NMR analyses of the [α-^{15}N]Lys-labeled PLC-δ1 PH domain. The ^{1}H-^{15}N HSQC NMR spectra without (black) and with (red) IP_3. (inset) The one-dimensional ^{15}N projections of the HSQC NMR spectra around the Lys-86 signal under the absence (black) and presence (red) of IP_3.

2. Solid State ^{95}Mo NMR of Paramagnetic Crystals of Polyoxomolybdates

For a group 6 element of molybdenum, there exist stable compounds with all valences from Mo(0) to Mo(VI). Among them, Mo(V) species are included in materials such as ε-Keggin anion and nanosized oxides with ring-, tube- and ball-structure. Localization of the d^1 electron in Mo(V) has attracted attention in terms of molecular design and solid state physics like optic, electric and magnetic properties. Since ^{95}Mo is a quadrupole nuclei of spin $I = 5/2$ with the small gyromagnetic ratio $\gamma = -1.743 \times 10^7$ rad s^{-1} T^{-1}, it is difficult to measure ^{95}Mo NMR spectra in solid state in general. Recently, we have reported solid-state ^{95}Mo NMR spectra of mixed balanced polyoxomolybdates(V, VI) with the localized or delocalized d^1 electrons measured by enhancing sensitivity and resolution using a high-field magnet.[3] Furthermore, we clarified a disorder structure of $[PMo_{12}O_{36}(OH)_4\{La(H_2O)_{2.75}Cl_{1.25}\}_4]27H_2O$ with the ε-Keggin $\{Mo_{12}\}$ core by ^{95}Mo NMR in solids.

In this study, we investigated a paramagnetic polyoxomolybdate of $[Mo_{12}O_{30}(OH)_{10}H_2\{Ni(H_2O)_3\}_4]$ (hereafter, abbreviated as $\{Mo_{12}\}$(Ni)). Although the core of this crystal is also ε-Keggin $\{Mo_{12}\}$, it is caped with four paramagnetic $Ni(II)(H_2O)_3$. In order to examine coupling constants appearing in solid state NMR, we measured high-field ^{95}Mo NMR in solids, simulated the obtained spectra numerically, and performed DFT calculation.

Figures 3(i-a) and 3(ii-a) show the ^{95}Mo NMR static spectra of $\{Mo_{12}\}$(Ni) measured under 21.8 and 11.7 T, respectively. Considerably broadened spectra with breadth of several thousands of ppm were obtained under both magnetic fields. In the spectral simulation, in addition to the quadrupole and chemical shift interactions, the hyperfine interaction between ^{95}Mo nuclei and unpaired electron spins in paramagnetic Ni(II) ions were considered as internal interactions. The simulated spectra shown in Figures 3(ii-a) and 3(ii-b) represented experimental spectra well. Since the broadening due to the quadrupole and anisotropic chemical shift interactions were quite large, the effect of the anisotropic hyperfine interaction on the spectral lineshape was small. In order to investigate the contribution from the isotropic hyperfine interaction, the ^{95}Mo NMR spectrum was measured at 173 K under 11.7 T (Figure 3(i-c)). Notable difference of the spectra between 301 and 173 K was not found. By lowering of temperature, although the line width was slightly broadened owing to the increase of magnetization of the electron spins, we needed not consider temperature dependence of the isotropic shift in the simulation.

The coupling constants for the internal spin interactions were also obtained from DFT calculation. Although the chemical shift estimated by DFT was larger than that by the spectral simulation, the trend such as a considerably large anisotropic chemical shift agreed between DFT and spectral simulation. Also, a spin density localized around the Ni(II) ions was obtained by DFT calculation, which agreed with small temperature-dependence of the whole shift of the spectra.

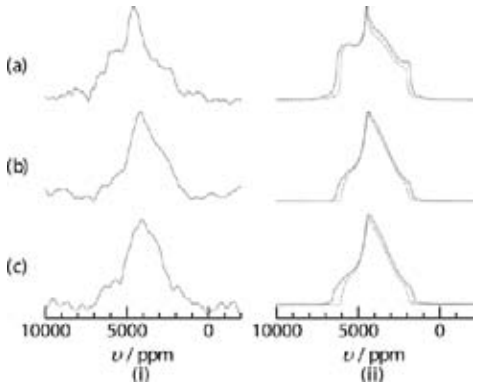

Figure 3. ^{95}Mo NMR static spectra of $\{Mo_{12}\}$(Ni) under the magnetic field of (a) 21.8 and (b, c) 11.7 T. (b) and (c) show the spectra at 301 and 173 K, respectively. (i) and (ii) show the observed and simulated spectra, respectively. The solid and broken lines in (ii) show the theoretical curves with and without considering the hyperfine interaction, respectively.

References

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3) T. Iijima, T. Yamase, M. Tansho, T. Shimizu and K. Nishimura, *Chem. Phys. Lett.* **487**, 232–236 (2010).