Solid State NMR for Molecular Science

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

1. NMR Analysis of Intramolecular Allostery in the Phosopholipase C-δ1 Pleckstrin Homology Domain

Proteins are generally activated by interactions with ligands, such as proteins, lipids, peptides, nucleotides, ions, photons, odorants, or other chemical compounds. Ligand binding usually induces changes in the protein conformation and dynamics, which also occur at distal sites away from the ligand-binding site in the protein molecule through intramolecular signal transductions, causing allosteric regulation of protein functions. Although elucidation of allosteric mechanisms is expected to be useful for regulations of protein functions and for allosteric drug designs, the detailed molecular mechanisms remain unclear.

Our previous study suggested the existence of intramolecular allosteric interactions in the phospholipase C (PLC)-δ1 pleckstrin homology (PH) domain.[1] The PLC-δ1 PH domain binds to phosphatidylinositol 4,5-bisphosphate (PIP_2) in the cell membrane, and inositol 1,4,5-triphosphate (IP_3), a product of PIP_2 hydrolysis by PLC-δ1. Mutational analyses of the PLC-δ1 PH domain demonstrated that conformational disruption of the characteristic short α-helix (α2) from residues 82 to 87 results in reduced affinity for IP_3 and in thermal instability, and that the phenyl ring of Phe-87 contributes to effective stabilization of the IP_3-binding state.[1] However, the α2-helix does not make direct contact with IP_3 in the crystal structure of the PLC-δ1 PH domain complexed with IP_3, and our findings therefore indicate that the α2-helix indirectly interacts with the IP_3-binding site through intramolecular allosteric interactions. In this study, we investigated the detailed molecular mechanisms of intramolecular allosteric interactions among spatially separated sites in the PLC-δ1 PH domain by using NMR.[2]

To detect the local environmental changes in the protein induced by ligand binding and site-specific mutations, we analyzed the ^{1}H-^{15}N HSQC NMR spectra of selectively [α-^{15}N]Lys-labeled PLC-δ1 PH domain and its mutants in the presence and absence of IP_3. In the wild-type protein, IP_3-dependent chemical shift changes were observed for all lysine signals, indicating that IP_3 binding affects the local environments at all lysine residues. Chemical shift perturbation (CSP) analyses for the wild-type protein demonstrated that more

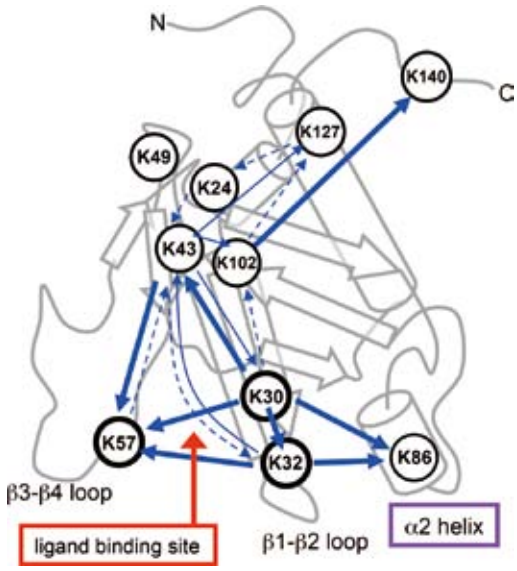

Figure 1. Graphical summary of the mutational effects on the PLC-δ1 PH domain. The bold, thin and dashed blue arrows indicate directions from mutated residues to affected residues with major, medium and minor CSP changes, respectively.

marked changes of chemical shifts were observed for Lys-43, Lys-102, and Lys-127, of which the α-nitrogen atoms are located far from the IP_3-binding site consisting of Lys-30, Lys-32, and Lys-57, suggesting that more drastic changes occurs at these distal sites on IP_3 binding.

If specific IP_3 binding induces local environmental changes at distal sites, mutations at the ligand-binding site would also affect local environments at spatially separated sites in the PLC-δ1 PH domain. The mutational analyses for signal assignments demonstrated that the single lysine mutants, K30A, K32A, K43A and K102A, showed significant chemical shift changes of at least two signals with drastic CSP changes as compared with the lysine chemical shifts of the wild-type protein under the ligand-free state. The effects of single lysine mutations on the other lysine residues are graphically summarized in Figure 1. These results indicate that an interaction network mainly consisting of the side chains of Lys-30, Lys-32, and Lys-43, but not Lys-57 or Lys-86, exists in the ligand-free protein.

The IP_3 titration experiment of the wild-type protein also demonstrated that in the ligand-free state, the α2-helix undergoes intermediate chemical exchange between at least two conformations with different population, and that IP_3 binding stabilizes one of the two conformations.[2] Interestingly, such stabilization of the α2-helix (Lys-86) induced by IP_3 binding was also observed in F87Y, but not in K57A or F87A (Figure 2) , indicating that the side chains of Lys-57 and Phe-87 contribute to stabilization of the IP_3-binding state, although Lys-57 does not contribute to the interaction network in the ligand-free state. Our results therefore strongly suggested that the pre-existing interaction network, mainly consisting of Lys-30, Lys-32 and Lys-43, in the ligand-free state is modified by IP_3 binding, resulting in formation of a new interaction network, in which Lys-57 and Phe-87 contribute to stabilization of IP_3-binding state.[2]

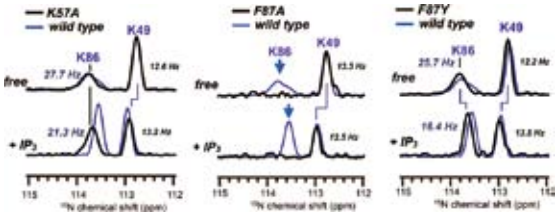

Figure 2. ^{15}N projections around the Lys-86 signal of the ^{1}H-^{15}N HSQC NMR spectra of [α-^{15}N]labeled K57A (left), F87A (middle) and F87Y (right) in the absence (top) and presence of IP_3 (bottom) as compared with those of the wild-type protein (blue).

2. A DFT Study of Electron Absorption of Polyoxomolybdates with d^1 Electrons

Among molybdenum with an integer valence from Mo^0 to Mo^{VI}, many Mo^V species are included in polyoxomolybdates of ε-Keggin anions and nano-sized oxides with characteristic shape. Localization of d^1 electrons of the Mo^V species has attracted much attention in terms of molecular design and solid state physics. Recently, we have found from solid-state ^{95}Mo NMR and DFT calculation that for $\{Mo_{12}(La)\}$ ($[PMo_{12}O_{36}(OH)_4\{La(H_2O)_{2.75}Cl_{1.25}\}_4]\cdot 27H_2O$) having eight d^1 electrons in a molecule, (i) there are two different molybdenum sites with population of 2:1 and (ii) they are basically corresponding to the species of Mo^V and Mo^{VI}, but some of the d^1 electrons are delocalized. This is a somewhat surprising result, because no intervalence charge-transfer bands for Mo^V to Mo^{VI} have been observed so far. In this work, we calculated electron absorption spectra of some polyoxomolybdates including $\{Mo_{12}(La)\}$ by DFT.

DFT calculation of the absorption spectra was implemented by the ADF2013.01 software. The local density approximation of VWM augmented with the Becke-Perdew GGA was employed for the exchange-correlation functional. The TZ2P or DZ all-electron basis set was used. Only allowed transitions were used for calculation of the absorption spectra.

Figure 3 shows the electron absorption spectra of polyoxomolybdates in the range of wavelength 400–1,400 nm obtained by DFT. Figure 3(i) is the spectrum of $\{Mo_{16}\}$ ($[Me_3NH]_6[H_2Mo^V_{12}O_{28}(OH)_{12}(Mo^{VI}O_3)_4]\cdot 2H_2O$), where twelve d^1 electrons are localized to form six Mo^V–Mo^V bonds of the ε-Keggin core. The relatively strong peak is only at around 430 nm that explains the brown color of this crystal. The weak peaks at ca. 590 and 655 nm are due to the d–d transition of the d^1 electrons of Mo^V. Figure 3(ii) shows the electron absorption spectrum of $\{Mo_{12}(La)\}$. It is found that the number of the peak increased both in the visible-light and near-infrared regions for $\{Mo_{12}(La)\}$. However, the absorption with short-wavelength visible light is consistent with the bark color of the crystal. Also, the intensity of the increased d–d transitions with wavelength of the near-infrared region would be not enough for the electron transition to be observed. Therefore, the slight delocalization of the d^1 electrons of $\{Mo_{12}(La)\}$ seems to be plausible.

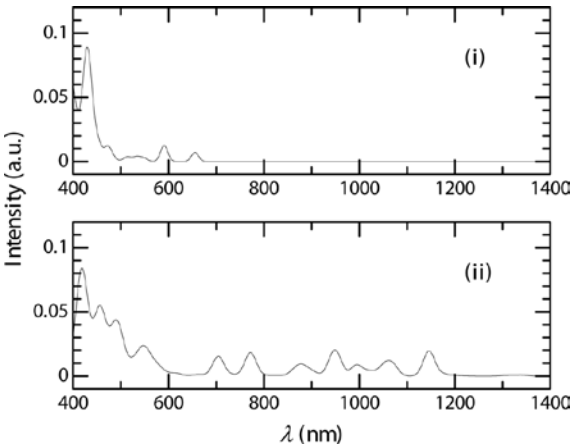

Figure 3. Electron absorption spectra for the polyoxomolybdates of (i) $\{Mo_{16}\}$ and (ii) $\{Mo_{12}(La)\}$ obtained by DFT calculation.

References

1) M. Tanio and K. Nishimura, *Anal. Biochem.* **431**, 106–114 (2012).
2) M. Tanio and K. Nishimura, *Biochim. Biophys. Acta* **1834**, 1034–1043 (2013).