2P049**NMR study on the interaction of the transactivation domain of ATF-2 with MAP kinase p38**

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To investigate the mechanism of transcriptional regulation of activating transcription factor-2 (ATF-2), we have examined the direct interaction between the transactivation domain (TAD) and the DNA binding domain (DBD) of ATF-2 or p38 Mitogen-activated protein kinase (MAPK) by using NMR techniques. The ATF-2-TAD contains TFIIIA-type Zn finger motif in N-terminal and extended structure in C-terminal, while C-terminal region has phosphorylation motif (TP repeat). MAP kinases are activated in response to a diverse array of extra-cellular stimulus from growth factors and cytokines to environmental stress factor. To study the structure of ATF-2-TAD bound to DBD, we performed NMR experiments. Thus, residues 83 to 90 of TAD, and even residues flanking this region, adopt the α -helical structure when bound to DBD. The largest deviations are clustered in the C-subdomain of TAD, which are clearly different from the locations of maximal chemical shift deviations observed upon complex formation between p38 α and TAD. The results show that the interaction site of the ATF-2 TAD is different from in inactive or active form. In conclusion, the ATF-2 DBD interacted with the C-subdomain in inactive, while p38 α interacted with the N-subdomain of ATF-2 TAD in active. Our NMR studies provide that phosphorylation disrupts the TAD and DBD interaction. Consistent with this possibility, phosphorylation of ATF-2 by p38 α kinase dramatically increases its *in vitro* DNA-binding activity.

2P051**Differences between solution and crystal structures of a DNA binding protein**

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Protein structures solved by X-ray and NMR are each determined in entirely different environments. However, it is difficult to obtain a clear-cut explanation of the differences between the two types of structures, because the lower quality of NMR structures tends to prevent from a rigorous comparison. Here, by refining the NMR structure using elaborated simulation techniques, we tried to analyze the differences between NMR and crystal structures of a protein-DNA complex. In this study, we supplemented the experimental information of distance and dihedral restraints with the force field used in molecular dynamics simulations, which includes the fully charged protein and DNA, and explicit water molecules, as well as the proper treatment of electrostatic interactions. In addition, we used simulated annealing (SA) to search a vast conformational space for most favorable structure. The system considered here is PhoB DNA binding domain complexed with DNA. After refining the NMR structure, we compared the resultant structure with the crystal structures of four PhoB-DNA complexes contained in an asymmetrical unit of the crystal. In the analysis, we focused on hydrogen bonding and side-chain conformations in the PhoB-DNA interface, and hydrogen bonding with water. As the results, we found that the refined NMR structure actually resembles the crystal structure more than observed in the NMR structure obtained by the conventional method.

2P050**Structural Analysis of G-Protein-Bound Mastoparan-X by Solid-State NMR**○Makiko Hori¹, Yasuto Todokoro¹, Ikuko Yumen¹, Toshiyuki Kohno², Kaori Wakamatsu³, Toshimichi Fujiwara¹, Hideo Akutsu¹¹Institute for Protein Research, Osaka University, Suita, ²Mitsubishi Kagaku Institute of Life Sciences (MITILS), Machida, ³Department of Biochemical and Chemical Engineering, Gunma University, Kiryu

Mastoparan-X (MP-X) isolated from wasp venom is an amphiphilic peptide which activates GTP-binding regulatory protein (G-protein). The conformation of MP-X bound to G-protein was determined by solution NMR. However the interaction sites between MP-X and G-protein are not determined. Solid-state NMR allowed the determination of membrane-bound MP-X structure and the detailed analysis of the interaction with bilayer membranes. Thus solid-state NMR should also be useful for determining the G-protein-bound MP-X structure and probing the interaction with G-protein. Here we have conducted magic-angle spinning solid-state NMR to reveal structure and the interaction sites. We have determined the secondary structure of uniformly ¹³C, ¹⁵N labeled MP-X bound to the deuterated α subunit of G₁₁ protein by solid-state NMR. MP-X was dissolved in an NMR sample buffer including G₁₁ α . Then we added to the solution 30 times as much trehalose as G₁₁ α in molar ratio as a cryoprotectant. Most NMR spectra were recorded on a solid-state NMR spectrometer at a static field of 11.8 T. 2D broadband ¹³C-¹³C correlation spectra were obtained with a spin-diffusion pulse sequence (DARR) at a mixing time of 10 ms. This mixing scheme provides intra-residue correlations. The chemical shifts of C γ , C α and C β suggested that the G₁₁ α -bound MP-X also forms an amphiphilic α -helix from W3 to C-terminal L14. Agreements of these chemical shifts with those for membrane-bound MP-X indicate the similarity between G-protein- and membrane-bound structures.

2P052**Solution Structure of Variable-type Domain of Receptor for Advanced Glycation Endproducts**○Shigeyuki Matsumoto¹, Takuya Yoshida¹, Hiroko Murata¹, Hiroshi Yamamoto², Yuji Kobayashi^{1,3}, Tadayasu Ohkubo¹¹Grad. Sch. of Pharm. Sci., Osaka Univ., ²Grad. Sch. of Med. Sci., Kanazawa Univ., ³Osaka Univ. of Pharm. Sci.

Diabetes is defined by a chronic hyperglycemia. Advanced glycation endproducts (AGE) resulting from a Maillard reaction increase in diabetic patients and cause both macrovascular and microvascular disorders which result in diabetic complications. The receptor (RAGE) has been identified to be a single-span transmembrane protein belonging to the immunoglobulin superfamily. Due to the clinical significances of the vascular complications, RAGE is currently focused as an attractive target for drug discovery of candidates which inhibit the intracellular signaling by interfering the AGE-RAGE binding. We have determined here by NMR the three-dimensional structure of the variable-type domain of RAGE (vRAGE), which has been revealed to contain the binding site of AGE. In solution, vRAGE forms a β sandwich structure composed of seven β -strands and three complementarity-determining region (CDR) -like loops. The overall structure exhibited a typical V-type immunoglobulin fold with an additional short α -helix. It is characteristic that almost half of the molecular surface is covered by positively charged residues. This positive region should contribute to the binding of negatively charged AGE. It was found that two strands among nine β -strands in the V-type immunoglobulin fold are flexible. This might be implicated with another feature of RAGE that has binding ability to various ligands as a multiligand receptor related to various diseases, such as inflammation, Alzheimer's disease and tumors.