Chapter VI

General Conclusion

My structural studies on Ca^{2+}/CaM molecular recognition processes can be summarized as follows:

(I) **Solution structure of Ca^{2+}/CaM complexed with its binding domain from rat CaMKK reveals a novel mode of molecular recognition.**

(a) CaMKK peptide adopts an α-helix structure followed by a hairpin-like loop, which enables the recognition of the longer region by Ca^{2+}/CaM.

(b) The C-terminal residue, Phe 463, interacts with both CaM and CaMKK peptide itself. The importance of Phe 463 is confirmed by the site-directed mutagenesis experiment carried out by Tokumitsu *et al.*

(c) CaMKK peptide binds to CaM in the reverse orientation compared with those in the other CaM-target peptide complexes. The binding orientation of the target peptide seems to be determined by the position of the cluster of basic residues in its sequence.

(d) Comparison of the structure with other complexes suggests that the relative orientation of both CaM domains is optimized to make more interactions with the target, which is achieved by the high flexibility of the domain linker and high homology between both CaM domains.

(II) **Solution structure of Ca^{2+}/CaM - W-7 complex reveals the basis of diversity in molecular recognition of the hydrophobic pocket of each domain of Ca^{2+}/CaM.**

(a) One W-7 molecule binds to the hydrophobic pocket in each CaM domain.

(b) In each domain, the W-7 chloronaphthalene group interacts with four methionine methyl groups and other aliphatic and aromatic side-chain in a deep hydrophobic pocket.

(c) The orientation of W-7 ring differs significantly from those of the

Trp 800 indole ring of smMLCK and the phenothiazine ring of TFP.

(d) The rearrangement of side-chains such as Phe 19/Phe 92, Met 36/Met 109, and Met 72/Met 145 upon W-7 binding maximize the van der Waals contacts with W-7, suggesting that this is likely a general mechanism to accommodate various aromatic rings.

(III) Globular structure of Ca^{2+}/CaM is induced by W-7 binding in solution.

(a) Binding of two W-7 molecules induces a globular shape for Ca^{2+}/CaM, probably caused by an inter-domain compaction.

(b) Ca^{2+}/CaM has a tendency to form a globular structure in solution, which is inducible by a small compound like W-7.

(IV) Symmetric covalent linkage of W-7 results in novel derivatives with increased inhibitory activities against Ca^{2+}/CaM complex.

(a) Structure-based design successfully provided the novel CaM antagonist with higher activity than W-7.

(b) The interactions of the designed compound with Ca^{2+}/CaM seems essentially the same with those of W-7, a result confirmed by NMR spectral change.

(c) The increase of the inhibitory activity can be due to the increased efficiency of the binding of the second chloronaphthalene group of W-7.

In the chapter III, I showed that the binding of two W-7 molecules induces a globular conformation for Ca^{2+}/CaM in solution. Although the globular structure is achieved by the compaction of both CaM domains, no structural information was obtained for the relative orientation of both

domains, suggesting that the both domains are not always fixed to each other. However, in the chapter IV, I also demonstrated that the interaction of Ca^{2+}/CaM with a molecule of the covalently linked derivative of W-7, $(W\text{-}7)_2$, is similar to that with two W-7 molecules, in which each of the two chloronaphthalene groups in a $(W\text{-}7)_2$ molecule is inserted to the hydrophobic pocket of CaM each domain. In the Ca^{2+}/CaM-$(W\text{-}7)_2$ complex, the two domains of Ca^{2+}/CaM are linked by $(W\text{-}7)_2$, indicating that the hydrophobic surfaces of both CaM domains come close together in a globular form. Therefore, adoption of a globular structure upon the binding of CaM antagonists or target molecules seems a general mechanism in Ca^{2+}/CaM molecular recognition.

This tendency of CaM to adopt a globular conformation appears to be important for inducing a three-dimensional structure to target peptides, which cannot form a structure by themselves. Due to the local interaction between Ca^{2+}/CaM and the peptide, Ca^{2+}/CaM undergoes a structural change to a globular structure, in which the peptide is sandwiched by the hydrophobic surfaces of both CaM domains. In such hydrophobic environment, the helix conformation of the peptide with its hydrophobic side-chains towards outside could be induced and stabilized by the van der Waals interactions with Ca^{2+}/CaM.

Wintrode and Privalov reported that the binding of the MLCK peptide to Ca^{2+}/CaM is an enthalpy-driven process, in which van der Waals interactions dominantly stabilize the complex (Wintrode & Privalov, 1997). However, the relative orientation of the both domains are varied among the complexes, suggesting that van der Waals interactions between Ca^{2+}/CaM and target peptide is optimized by tuning the domain orientation. This could be achieved due to the high flexibility of the domain linker and the high adaptability of the hydrophobic pocket of each CaM domain.

Although electrostatic interaction is not dominant in the intermolecular

interactions, I proposed that the binding polarity of the target peptide could be mainly determined by the electrostatic interactions between the basic residues in the peptide and the glutamate cluster on the surface of Ca^{2+}/CaM. The dual directions of the peptide binding also contribute to the diversity in CaM molecular recognition.

Taken together, the diversity of molecular recognition by Ca^{2+}/CaM is achieved by the fine tuning of the relative orientation of the two domains and the high adaptability of the hydrophobic pocket in concert with the tendency to form a globular structure.