DA 2052(2) 1938

STRUCTURAL STUDIES ON CALMODULIN MOLECULAR RECOGNITION PROCESSES

Division of Applied Biochemistry Doctoral Program in Biotechnology University of Tsukuba

Masanori Osawa

CONTENTS

Contents						
Abbrevia	tions		V			
Chapter I	[General Introduction	1			
]	I-1	Calcium Signaling in Eukaryotic Cells	2			
]	I-2	Calmodulin as a Signal Transducer	2			
	I-3	Structure of Calmodulin	3			
	I-4	Molecular Recognition of Ca ²⁺ /CaM	4			
	I-5	The Aims of This Thesis	6			
	Figu	res & Tables	8			
Chapter :	ΙΪ	Solution Structure of Ca ²⁺ /CaM Complexed with its Binding				
Chapter II		Domain from Rat Ca ²⁺ /CaM Dependent Protein Kinase Kinase				
		Reveals a Novel Mode of Molecular Recognition	15			
	II-1	Summary	16			
	II-2 II-3	Introduction	17			
		Material and Method	20			
		II-3-1 Sample Preparation	20			
		II-3-2 NMR Spectroscopy	20			
		II-3-3 Structure Calculation	22			
		II-3-4 Site-directed Mutagenesis	23			
	II-4	Results and Discussion	24			
		II-4-1 NMR Spectroscopy	24			
		II-4-2 Structure Description	25			

		II-4-3 Variable Domain Orientation	26
		II-4-4 Peptide Polarity	27
		II-4-5 Correlation with Mutagenesis Studies	30
	II-5	Concluding Remarks	31
	Figu	res & Tables	32
Chapter	III	Solution Structure of Ca ²⁺ /CaM-W-7 Complex:	
		the Basis of Diversity in Molecular Recognition	46
	III-1	Summary	47
	III-2	Introduction	48
	III-3	Material and Method	50
		III-3-1 Sample Preparation	50
		III-3-2 NMR Spectroscopy	51
		III-3-3 Structure Calculation	51
	III-4	Results and Discussion	53
		III-4-1 NMR Spectral Change and Stoichiometry	53
		III-4-2 The Structure of Ca ²⁺ /CaM Complexed with W-7	54
		III-4-3 Comparison of W-7 and Target Peptide Bound to	
		Ca ²⁺ /CaM	56
		III-4-4 Comparison of the Ca ²⁺ /CaM Binding Mode	
		between W-7 and TFP	58
		III-4-5 Fine Tuning in CaM Conformation	59
	III-5	Concluding Remarks	62
	Figu	res & Tables	63

Chapter IV	Evidence for Calmodulin inter-domain Compaction in	
	Solution Induced by W-7 Binding	74
IV -1	Summary	75
IV-2	Introduction	76
IV-3	Material and Method	77
	IV-3-1 Sample Preparation	77
	IV-3-2 Small-angle X-ray Scattering	77
	IV-3-3 NMR Spectroscopy	78
IV-4	4 Results	80
	IV-4-1 The Radius of Gyration and the Distance Distribution	
	Function	80
	IV-4-2 NMR Spectral Changes	81
IV-5	5 Discussion	82
	IV-5-1 Globular Structure of Ca ²⁺ /CaM-W-7 Complex	82
	IV-5-2 Comparison with Globular Structure of Ca ²⁺ /CaM-TFP	
	Complex	83
	IV-5-3 Comparison with Ca ²⁺ /CaM-Target Peptide Complex	84
IV-6	6 Concluding Remarks	85
Figu	ires & Tables	86
Chapter V	Symmetric Covalent Linkage of W-7 Results in Novel	
1	Derivatives with Increased Inhibitory Activities against	
	Ca ²⁺ /CaM Complex	94
V-1	Summary	95
V-2	Introduction	96
V-3	Material and Method	99
	V-3-1 CaM Purification	99
	V-3-2 Chemical Synthesis of (W-7) ₂ Analogs	100

	*	V-3-3	In Vitro Protein Kinase Assays	104	
	7	V-3-4	Mass Spectrometry Measurements	105	
	7	V-3-5	NMR Spectroscopy	106	
	•	V-3 - 6	Miscellaneous	106	
V	-4 I	Results			
	•	V-4-1	Structures and Activities of the Novel (W-7) ₂		
			Derivatives	108	
	7	V-4 - 2	Comparison of the Ca ²⁺ /CaM Interaction between (W-7) ₂		
			and W-7	109	
V	-5 J	5 Discussion			
V	-6 (Concluding Remarks			
Fi	igure	es & Ta	ables	115	
Chapter V	I (Genera	l Conclusion	120	
Acknowledgments					
				127	
References					

Abbreviations

Ala, A: alanine Arg, R: arginine

Asn, N: asparagine Asp, D: aspartatic acid

Cys, C: cysteine Gln, Q: glutamine

Glu, E: glutamic acid Gly, G: glycine

His, H: histidine Ile, I: isoleucine

Leu, L: leucine Lys, K: lysine

Met, M: methionine Phe, F: phenylalanine

Pro, P: proline Ser, S: serine

Thr, T: threonine Trp, W: tryptophan

Tyr, Y: tyrosine Val, V: valine

CaM : calmodulin

apo CaM : calcium-unbound CaM

Ca²⁺/CaM : calcium-bound CaM

CaMK : Ca²⁺/CaM dependent protein kinase

CaMKI : Ca²⁺/CaM dependent protein kinase I

CaMKII : Ca²⁺/CaM dependent protein kinase II

CaMKIV : Ca²⁺/CaM dependent protein kinase IV

CaMKK : Ca²⁺/CaM dependent protein kinase kinase

MLCK : myosin light chain kinase

skMLCK : skeletal muscle MLCK

smMLCK: smooth muscle MLCK

NMR : nuclear magnetic resonance

NOE : nuclear Overhauser enhancement

NOESY : NOE spectroscopy

HSQC : heteronuclear single quantum coherence

CT-HSQC : constant time HSQC

R.M.S.D.: root mean square deviation

W-7 : N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide

TFP: trifluoperazine

J-8 : N-(8-aminooctyl)-5-iodo-1-naphthalenesulfonamide

SAXS : small-angle X-ray scattering

 R_g : radius of gyration

p(r): pair distance distribution function

(W-7)₂ : covalently linked analog of W-7 dimer