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CRYSTAL STRUCTURES OF CYCLOPHILIN AND ITS PARTNERS

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1. ABSTRACT

Cyclophilin (CyP) is a cytosolic receptor of immunosuppressive drug cyclosporin A (CsA). The binary complex of CyP-CsA inhibits the activity of Ca^{2+}/calmodulin dependent serine/threonine calcineurin (CN). The inhibition of CN in turn disables the transcription activity of nuclear factor of activated T cell, thus suppressing the T cell activation and cardiac hypertrophy. CyP is also an enzyme catalyzing peptidyl-prolyl *cis-trans* isomerization and serves as a molecular chaperone in various biological processes. For example, CyPA is involved in the assembly/deassembly of HIV-1 virion and is required for the full infectious activity of HIV-1. However, the *in vivo* function of CyP remains a mystery. This review will describe the three-dimensional structures of CyPs and its partners and discuss the structural clues to understanding the CyP functions in biological processes. The structures of CyP in complex with proline-containing peptides provided insight into the mechanism of peptidyl-prolyl *cis-trans* isomerization. The structures of CyPA in complex with HIV-1 capsid protein and its peptides revealed details of interactions of CyP with HIV-1 capsid protein, thus providing a guideline for design of anti-HIV drugs. The rearrangement of two tetratricopeptide repeats of the "large cyclophilin" CyP40 into a long helix under the crystallization conditions might be biologically relevant to the CyP40 function in the hsp90 molecular chaperone system. The structures of the binary CyPA-CsA and ternary CN-CyPA-CsA complexes showed how CsA binds to its receptors and therefore provide a template for design of new immunosuppressive drugs.

2. INTRODUCTION

Cyclophilin (CyP) is a family of cytosolic proteins involved in many biological processes. As a family of immunophilin, CyP binds the immunosuppressive drug cyclosporin A (CsA) (1). The complex of CyP-CsA in turn inhibits calcineurin (CN), a Ca^{2+}/calmodulin dependent serine/threonine phosphatase (PP2B) (2, 3). The inhibition of the dephosphorylation activity of CN turns off the transcription activity of nuclear factor of activated T cell (NFAT), thus suppressing the signal transduction toward the T cell activation (4) and the cardiac hypertrophy (5-7). On the other hand, CyP is an enzyme that catalyzes peptidyl-prolyl *cis-trans* isomerization (PPIase) (8, 9). Since a correct conformation of proline, which is generated by the peptidyl prolyl *cis-trans* isomerization, is a rate-determining step in protein folding, CyP is assumed to be a foldase (10). In addition, CyP is thought to serve as a molecular chaperone in several unrelated biological processes. For example, cyclophilin 40 (CyP40) is a member of the hsp90 molecular chaperone system in regulation of steroid hormone receptor. The CyP homologues, *Nina*A in complex with rhodopsin (11) and RanBP in complex with red/green opsin (12), presumably serve as molecular chaperones in the phototransduction pathway. Last, CyP binds HIV-1 capsid protein and is required for the full infectious activity of HIV-1 (13-15). In spite of the important roles of CyPs in various biological processes, the *in vivo* function of CyP remains a mystery. This review will focus on the structures of CyPs and its partners (Table 1), and discuss the structural clues to the functions of cyclophilins.

3. CYCLOPHILINS

Over three hundred sequences of cyclophilins from bacteria to human (55) can be generally classified into two categories: "small" and "large" CyPs. The small CyPs contain a single domain called the PPIase domain that has an *in vitro* catalytic activity of peptidyl-prolyl *cis-trans* isomerization. The large CyPs consist of a PPIase domain

Table 1. Structures of cyclophilins and its partners

Proteins	Ligands (PDB codes)	References
Unligated CyP		
Human CyPA	(2CPL, 1OCA)	16-18
Human Nucl CyP20	(1QOI)	19
Human CyP40	(1IHG, 1IIP)	20
B. Malayi CyP	(1A33, 1A58)	21, 22
C. Elagan CyP, CyPB	(1DYW, 1HOP)	23, 24
E coli CyP, CyPB (K163T)	(1CLH, 1J2A)	25
Yeast CyPA(Cpr1)	(1IST)	
CyP in complex CsA or its derivatives		
Human CyPA	CsA (1CWA, 1CYS, 2RMA)	26-30
	$MeBMT_2$-CsA (2RMB, 1CWB)	30, 31
	2-Thr-CsA (1BCK), 2-Val-CsA (1CWF),	
	2-Val 3-(N-Methyl)-D-Ala-CsA (1CWI),	
	2-Val 3-S-Methyl-Sarcosine-CsA (1CWJ),	
	Mebmt2-Val3-D-(2-S-Methyl)Sarcosine-CsA (1CWK)	
	4 4-Hydroxy-Meleu-CsA (1CWL),	
	4 Meile-CsA 1(CWM), 3-D-Ser-CsA (1CWH)	32
	[4, N-Dimethylnorleucine]4-CsA (1CWC)	33
	Thr2, Leu5, D-Hiv8, Leu10-CsA (1CWO)	34
	(5-hydroxynorvaline)-2-CsA (1MIK)	35
	macrocyclic SFM (1NMK)	36
Human CyPB	[D-(Cholinylester)Ser8]-CsA (1CYN)	37
Murine CyPC	CsA, (2MRC)	38
P. Falciparum CyP & L120F, W171L mutants	CsA (1QNG, 1QNH)	39
B. Malayi CyP	CsA (1C5F)	40
E coli CyP, F112W mutant	CsA (1CSA)	41
CyP in complex with peptides		
Human CyPA	Ala-Ala-Pro-Ala, Ala-Ala-Pro-Phe (1RMH)	42, 43
	Ala-Pro (2CYH), Ser-Pro (3CYH), His-Pro (4CYH), Gly-Pro (5CYH)	44, 45
Human CyPH	U4/U6 Snrnp peptide (1MZW)	46
C. Elagan CyP	Ala-Pro (1E8K)	47
E coli CyP	Ala-Pro-Ala (1LOP)	48
CyP in complex with the N-terminal domain or peptides of HIV-1 capsid		
Human CyPA	HIV-1 CA domain (1AK4, 1M9C, 1M9D)	49
	H87A (1M9E), H87A, A88M (1M9F),	
	H87A, A88M, G89A (1M9X),	
	H87A, G89A (1M9Y)	52
Human CyPA	His-Ala-Gly-Pro-Ile-Ala (1AWQ, 1AWR)	
	His-Val-Gly-Pro-Ile-Ala (1AWU, 1AWV)	50
	25 residues of capsid p24 of HIV-1 (1FGL)	51
CyP-calcineurin complex		
Human CyPA-calcineurin	CsA (1M63, 1MF8)	**53, 54**

and one or more additional domains such as a tetratricopeptide repeat (TPR) domain in cyclophilin 40.

3.1. Small cyclophilin

Human cyclophilin A (CyPA), a cytoplasmic protein with a molecular weight of about 18 kDa, is a representative of "small" CyPs. The three-dimensional structure of CyPA was independently solved for the unligated CyPA (16) and CyPA in complex with a tetrapeptide N-acetyl-Ala-Ala-Pro-Ala-amidomethylcoumarin (42). The structure of CyPA is a beta-barrel with eight antiparallel beta-strands and two alpha-helices covering the bottom and top of the barrel (Figure 1). Inside the barrel is a hydrophobic core made up of residues Val6, Phe8, Ile10, Val20, Phe22, Leu24, Phe36, Leu39, Tyr48, Phe53, Ile56, Cys62, Leu98, Met100, Phe112, Ile114, Phe129, Val132, Val139, Met142, and Ile158 (17). A shallow pocket on the barrel surface is the active site for *cis-trans* isomerization of a peptidyl-prolyl amide bond. This pocket contains residues Arg55, Phe60, Met61, Gln63, Phe113, Trp121, Leu122, and His126.

3.2. Large cyclophilin

Cyclophilin 40 (CyP40), a member of "large" CyPs, is a co-chaperone of the hsp90 chaperone system for transformation of a steroid hormone receptor (56-58).

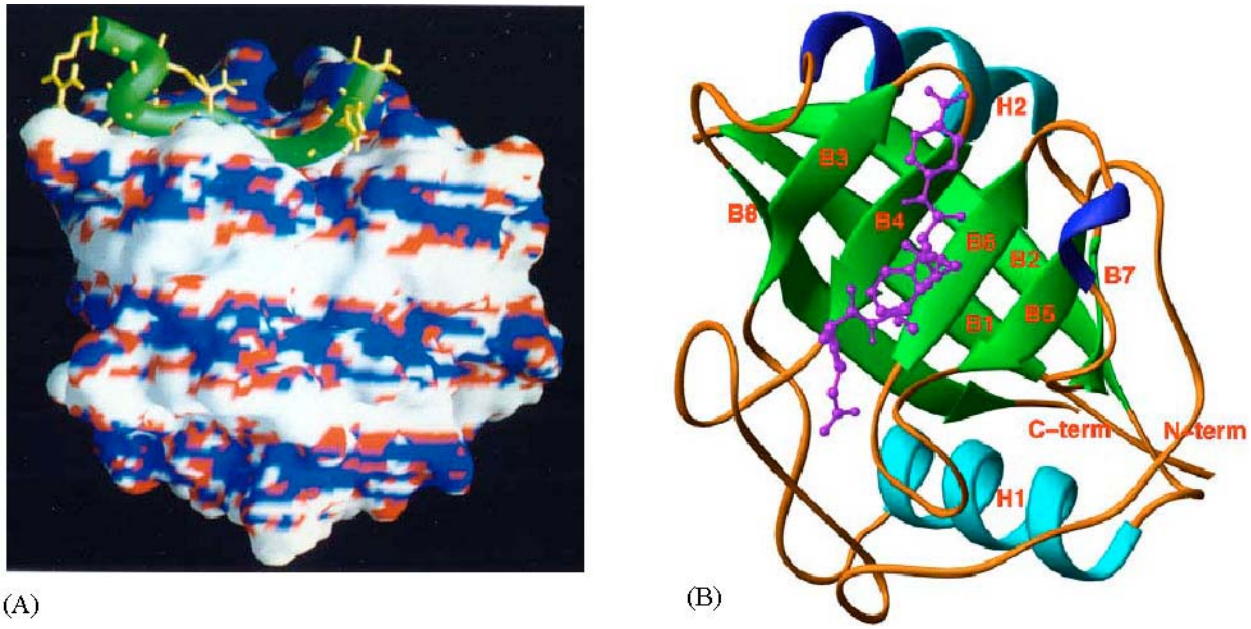

Figure 1. The CyPA structure. (A) Ribbon diagram of CyPA. A substrate is shown in purple ball-sticks. (B) Surface presentation of CyP in a view with 90^{o} rotations along the horizontal and then vertical axes. The green worm marks the binding of a ligand.

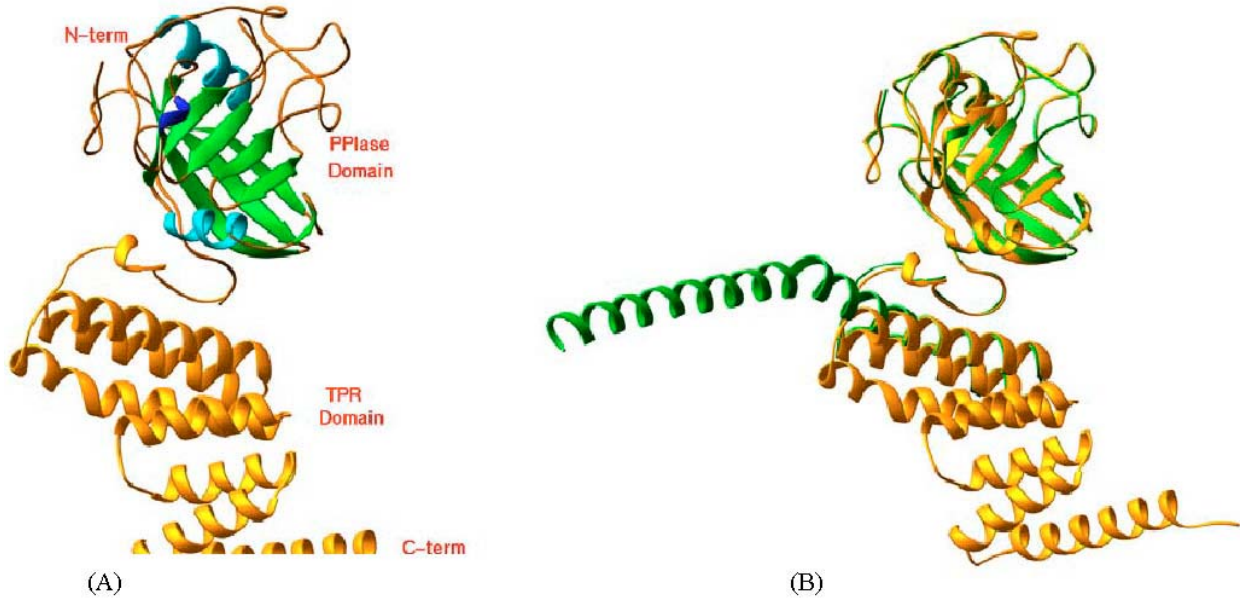

Figure 2. Ribbon presentation of CyP40. (A) The monomeric crystal of CyP40 showed typical foldings of PPIase domain (top) and three TPR units (golden). (B) A superposition between the tetragonal (green ribbons) and monomeric (golden) CyP40 crystal. The two C-terminal TPR units were refolded into a long helix.

CyP40 contains a PPIase domain at the N-terminus and a tetratricopeptide repeat (TPR) domain at the C-terminus (Figure 2). TPR is a structural motif frequently found in protein-protein interactions and contains about 34 amino acids arranged in two parallel alpha-helices (59, 60). CyP40 was crystallized in the monomeric and tetrameric forms (20). The PPIase domain in both crystal forms of CyP40 has the same folding as that of the "small" CyP, but its role in the hsp90 molecular chaperone system is unknown. The C-terminal TPR domain of CyP40 exists in two different conformations. The monomeric CyP40 crystal contains three TPR units that parallel packed against one another and are structurally similar to the hsp90-binding domains of protein phosphatase 5 (PP5) (61), FKBP51 (62), and Hop (Hsp70 and Hsp90 organizing protein) (63). Since the TPR domain of Hop binds the C-terminal sequences MEEVD of Hsp90 and IEEVD of Hsp70, it may be rational to predict the same binding of the CyP40 TPR domain to Hsp90 and Hsp70. However, the superposition of the TPR domains of Cyp40, FKBP51, PP5 and Hop showed variation of some important residues (62), implying that the binding

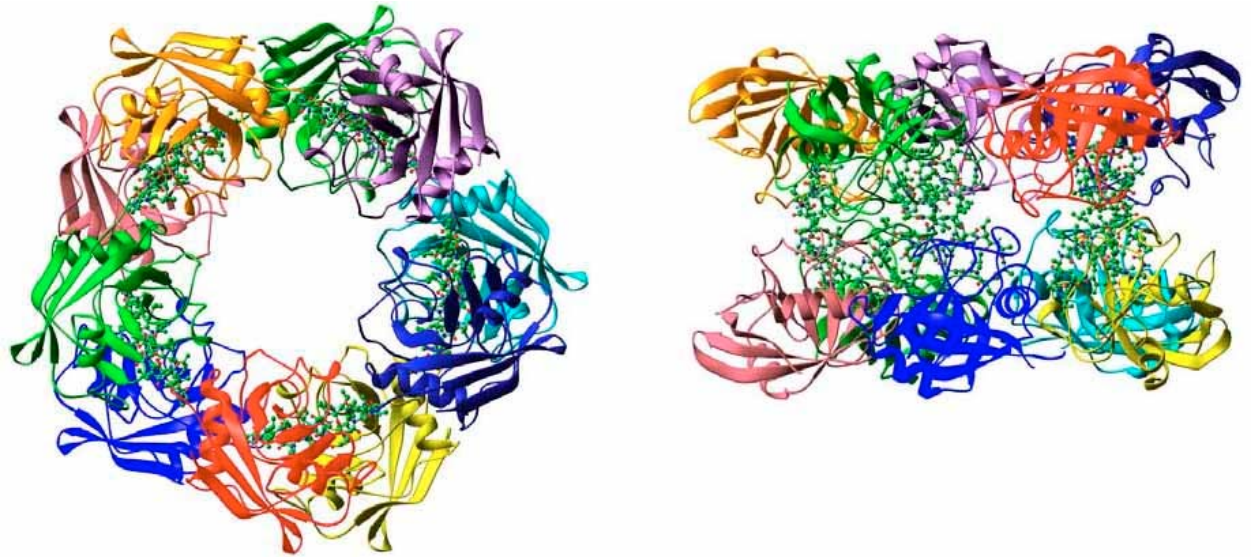

Figure 3. The decameric structure of CyPA-CsA. Each color of ribbons represents a molecule of CyPA. CsAs are shown in the ball-stick models. The right picture is a view of 90° rotation about the horizontal axis of the left picture.

modes of the TPR domains to Hsp90 would not be exactly the same. This argument is supported by the fact that the CyP40, FKBP51, and PP5, but not Hop contain a seventh helix extended beyond the last TPR motif. On the other hand, the two C-terminal TPR units in the monomeric crystal were refolded into a long helix in the tetragonal form of CyP40 (Figure 2). This conformational rearrangement is a unique structure character of CyP40 among known TPR containing proteins, and may be related to its chaperone activity, yet needs further studies.

4. CYCLIPHILIN IN COMPLEX WITH CYCLOSPORIN A

Cyclophilin and FKBP are two families of immunophilins for binding of immunosuppressive drugs CsA and FK506 (64, 65), respectively. Although CsA binding is not an intrinsic function of CyP because CsA does not naturally exist in human body, the potential use of the CyP-CsA structure information for design of new immunosuppressive drugs attracted intensive attention from both academic and industrial groups in the early 90s. Various crystal forms of CyPs in complex with CsA and CsA derivatives have been reported (Table 1). A typical packing of the CyPA-CsA complex is a decameric structure in which two pentamers of CyPA form the outer layers of a sandwich of CyPA-CsA-CsA-CyPA via both hydrophilic and hydrophobic interactions (Figure 3).

CsA binds to CyPA in a 1:1 stoichiometry. In a monomer of CyPA-CsA, six residues of MeBmt1, Abu2, Sar3, MeLeu9, MeLeu10, and MeVal11 of CsA interact with Arg55, Phe60, Met61, Gln63, Gly72, Ala101, Asn102, Ala103, Gln111, Phe113, Trp121, Leu122, and His126 of CyPA (30). Five hydrogen bonds are formed between MeBmt1 O of CsA and Gln63 NE2 of CyPA, Abu2 N and Asn102 O, MeLeu9 O and Trp121 NE1, MeLeu10 O and Arg55 NH1, and MeLeu10 and Arg55 NH2. In addition, CsA interacts with neighboring molecules of CyPA within the decamer and with CsA from the opposite pentamer via backbone contacts of Abu2, Sar3 and MeLeu4. However, the interactions between CyPA-CyPA or CsA-CsA in the decamer may be the consequences of crystallographic packing but not biologically relevant because the later study showed that a monomer of CyPA-CsA bind to calcineurin (53, 54). Nevertheless, the structural studies suggested that residues 3 and 8 of CsA are potentially the modification targets for improvement of water solubility without significant impact on the binding.

All peptide bonds of the bound CsA have the *trans* conformation in the structures of CyPA-CsA. One intramolecular hydrogen bond between the side chain hydroxyl group of MeBmt1 and the carbonyl oxygen of MeLeu4 is observed in the bound CsA of the NMR and X-ray structures. The conformation of CsA bound to CyPA is significantly different from CsA bound to Fab or in free solution (66), suggesting the adaptation of CsA for binding to the different receptors. However, the backbone conformation of CsA in the binary CyPA-CsA complex is very similar to that in the ternary CN-CyPA-CsA complex (53, 54).

5. CYCLOPHILIN AS A *CIS-TRANS* ISOMERASE

A peptidyl-prolyl *cis-trans* isomerase (PPIase) catalyzes *cis-trans* isomerization of a peptidyl-prolyl amide bond (67). Four families of PPIases have been identified: CyP, FKBP, parvulin, and Pin1, but share no sequence and structure similarity. CyP and FKBP are known as immunophilin involved in the T cell activation (68). Parvulin presumably functions in maturation and transportation of proteins (69). Pin1 is involved in regulation of cell mitosis (70) and contains a C-terminal PPIase domain and an N-terminal WW domain for specifically binding a phosphorylated Ser/Thr-proline motifs (71, 72).

The mechanism of *cis-trans* isomerization of a

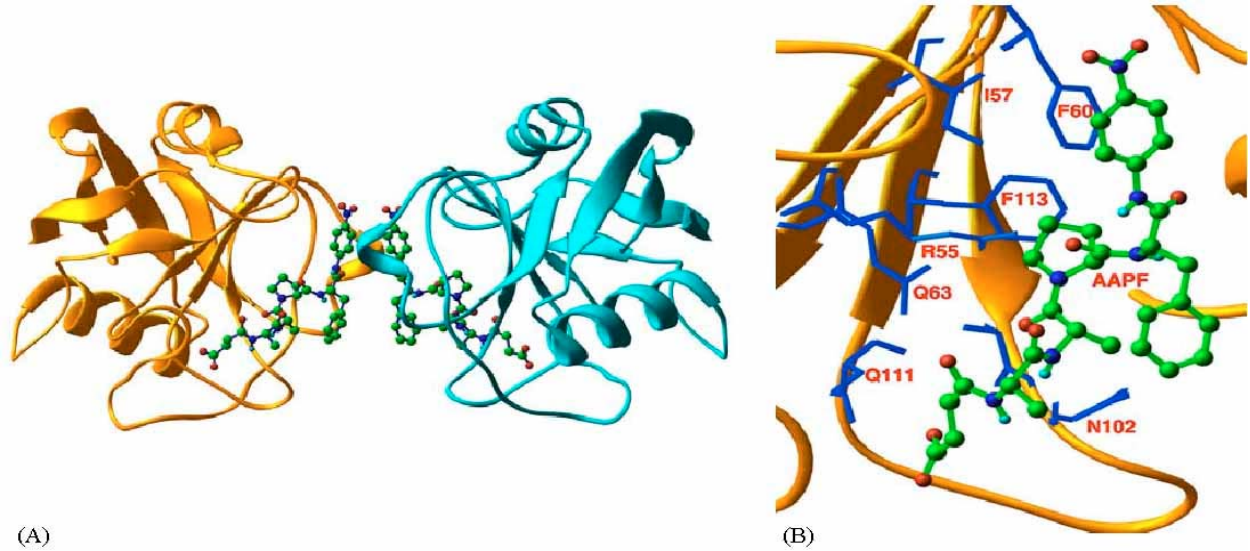

Figure 4. Binding of substrate succinyl-Ala-Ala-Pro-Phe-nitroaniline (AAPF). (A) Ribbon presentation of the CyPA dimer. AAPF is shown as ball-sticks. (B) Detailed interactions of AAPF (ball-sticks) with the CyP residues (blue sticks).

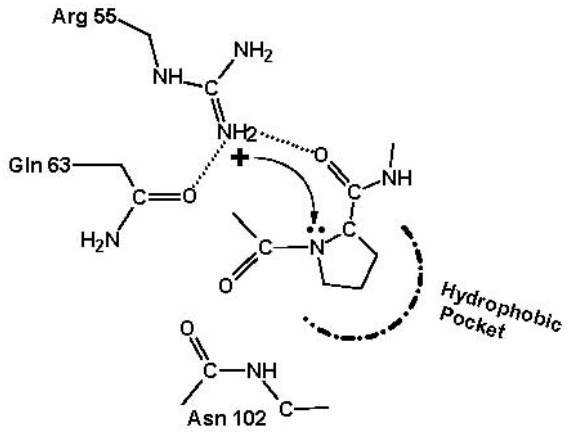

Figure 5. Schematic presentation of a putative mechanism for the peptidyl-prolyl *cis-trans* isomerization by CyP.

peptidyl-prolyl bond by CyP has been extensively studied by biochemical and structural approaches. "Catalysis by distortion" and "protonation on the amide nitrogen" are two most likely mechanisms. "Catalysis by distortion" proposes that the N-C=O peptide plane is distorted and stabilized by binding to CyP (73). The mechanism of "protonation on the amide bond" assumes that a serine or tyrosine protonates or forms a hydrogen bond with the amide nitrogen to deconjugate the N-C=O amide bond, on the basis of quantum chemistry calculations (74).

The crystal structure of CyPA in complex with substrate succinyl-Ala-Ala-Pro-Phe-nitroaniline (AAPF) shows that AAPF binds to the hydrophobic pocket on the surface of the CyPA barrel (43). AAPF interacts with the CyPA residues of Arg55, Ile57, Phe60, Met61, Gln63, Ala101, Asn102, Gln111, Phe113, Trp121, Leu122, and His126 (Figure 4). Four hydrogen bonds are formed between Ala2 N of AAPF and Asn102 O of CyPA, Pro3 O and Arg55 NH1, Pro3 O and Arg55 NH2, and Phe4 O and Trp121 NE1. In addition, the side chain of Arg148 of CyPA hydrogen-bonds to the nitro group of aniline of AAPF.

In the structure of CyPA-AAPF, the guanidine group of Arg55 forms a hydrogen bond with the carbonyl oxygen of proline of AAPF and is also located about 4 Å away from the prolyl nitrogen of AAPF. Although these interactions in the crystal structure represent a ground state, the positively charged guanidine nitrogen of Arg55 may dynamically approach the amide nitrogen during catalysis to interact with the lone pair electrons of the peptide nitrogen atoms (43, 75). As the result, the conjugation system of the peptide plane may be substantially weakened and the isomerization initiates. This putative mechanism is depictured in figure 5 and is consistent with the mechanism of "protonation on the amide nitrogen" (74).

As an enzyme, one would expect that both *cis* and *trans* conformers of a proline peptide substrate bind to the active site of CyP. However, most crystal structures of CyP in complex with dipeptides (44, 45), a tripepetide (48), or tetrapeptides (42, 43) showed the *cis* form of the proline peptides bound to CyP, except for the peptides of HIV-1 capsid protein, which can exist in either *trans* or *cis* conformation (49, 52). The crystal structures of CyPA in complex with wild type and five mutants of the N-terminal domain of HIV-1 capsid p24 showed that the *trans* prolyl peptides bind to CyP in a similar pattern as the *cis* peptides and the largest changes are the backbone conformations of two residues preceding the proline (52). On basis of the observation that the C-terminal portions of the *cis* and *trans* prolyl peptide bonds have the similar interactions and conformations, a pathway that the *cis-trans* isomerization propagates through the N-terminus of the peptide is proposed (52). However, an opposite pathway that the C-terminus undergoes conformational changes during the catalysis while the N-terminus is fixed was proposed on basis of the dynamic NMR experiments (76).

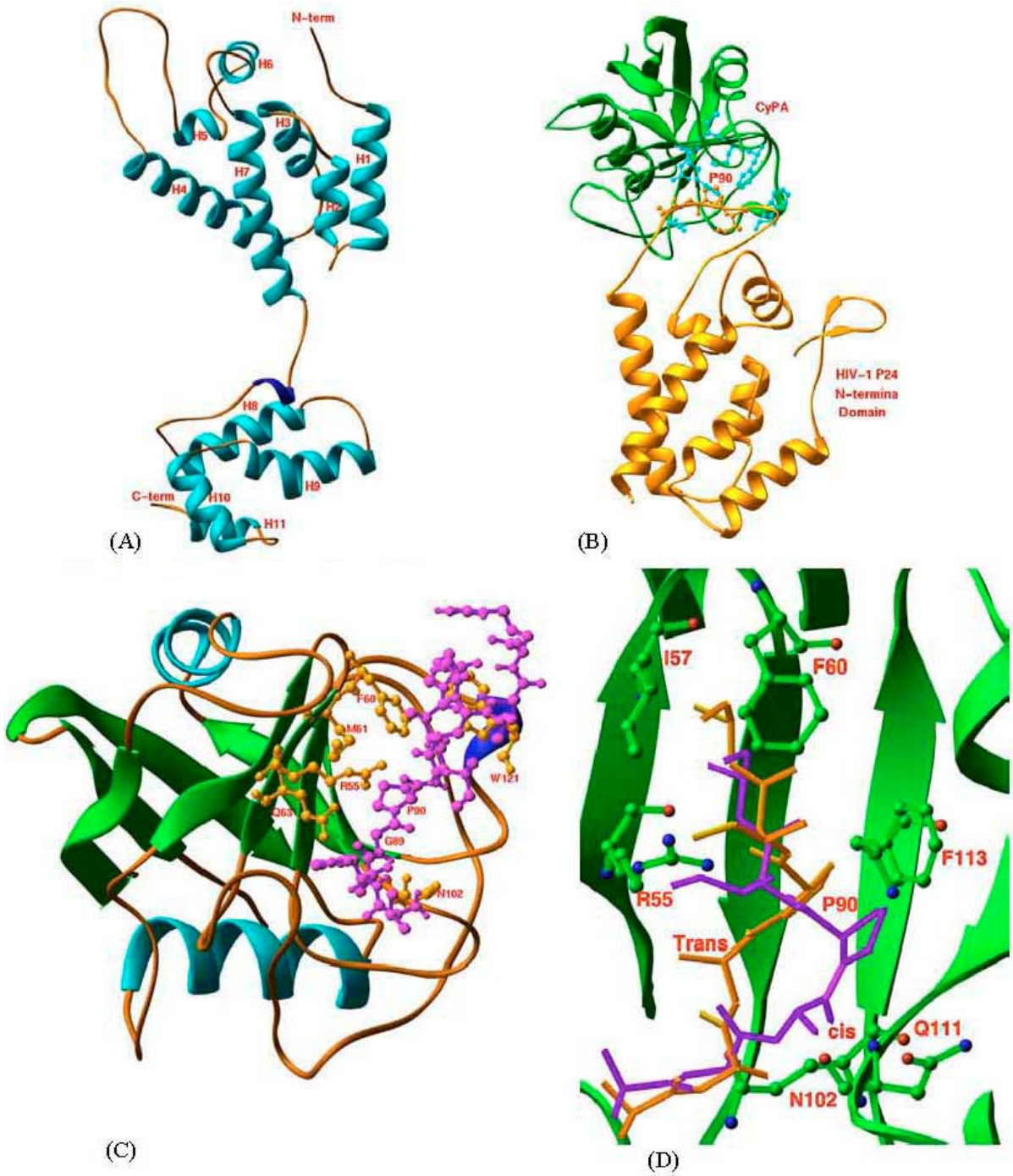

Figure 6. The structures of HIV-1 capsid protein and its complex with CyPA. (A) Ribbon presentation of HIV-1 capsid protein drawn with PDB entry 1E6J. (B) The complex of N-terminal domain of capsid p24 (golden ribbons) with CyPA (green). The CyPA residues for p24 binding are shown as the cyan balls. The p24 residues for binding of CyPA are shown as the golden balls. (C) A fragment of p24 (purple stick-balls) binds to CyPA. The CyP residues for p24 binding are shown as the golden balls. (D) Detailed view of the HIV-1 capsid peptides binding to CyPA (green ribbons). The capsid peptide in the *cis* configuration (purple sticks) is superimposed over the *trans* (golden) configuration. The CyPA residues are shown in ball-stick models.

6. CyPA IN COMPLEX WITH HIV-1 CAPSID PROTEIN P24

Capsid protein p24 (CA) is the coating protein of HIV-1 and oligomerizes to form the inner conical viral particle. The assembly of HIV virion particle is essential for understanding the HIV infectivity and for design of antivirus drugs, but is poorly understood (77, 78). The crystal structure showed that CA has two structure domains (Figure 6). The N-terminal domain of CA contains seven

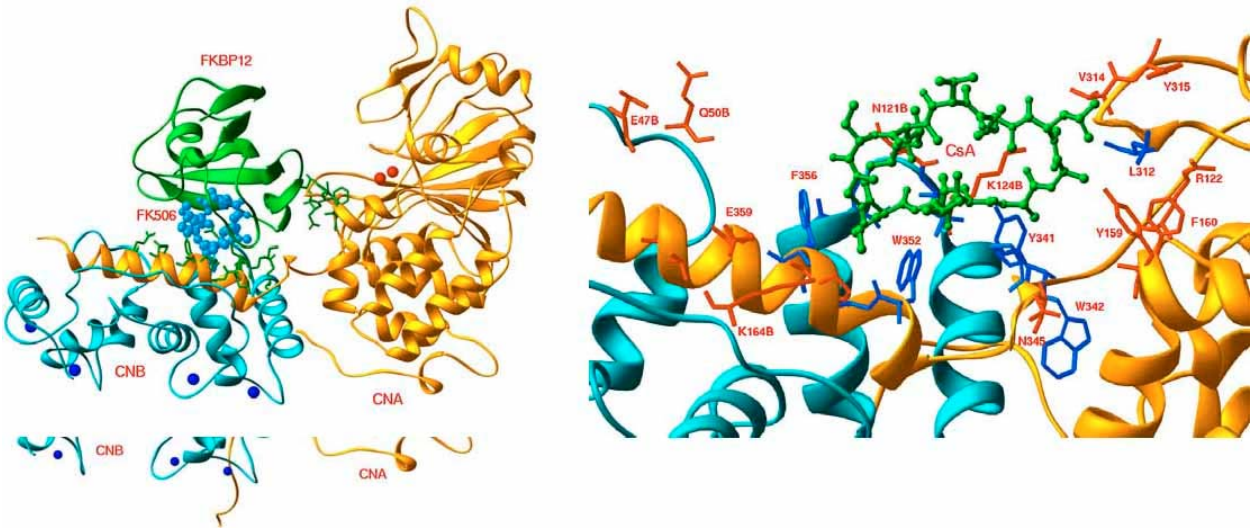

Figure 7. Ribbon presentation of calcineurin. (A) The CN-CyPA-CsA complex. CNA is shown in golden, CNB is in cyan and CyPA is in red. Green balls are CsA. Purple sticks represent the residues of CN for binding of CyPA-CsA. Red balls are the catalytic metals of Zn^{2+} and Fe^{3+}. The blue balls are calcium. (B) The CN-FKBP12-FK506 complex. FKBP12 is shown as green ribbons. FK506 is drawn as blue balls. The green sticks represent the residues of CN for FKBP12-FK506 binding. (C) CsA (green) binding to the CNA-CNB composite surface. The residues interacting with CsA are shown as blue sticks and residues for CyPA binding are in red.

helices, five of which arrange in a near parallel bundle and two short helices sit at the top of the structure (49, 79). The C-terminal domain of CA comprises four helices and is tightly associated into a dimer (80). Several models on how CA assembles into the conical core particle were proposed on basis of the structures and electron microscope (49, 80-83), but no one is predominantly convincing.

Cyclophilin binds to the N-terminal domain of HIV-1 CA and is required for full infectious activity of HIV-1 (13-15). The structure of CyPA in complex with the N-terminal domain of CA (49) or the peptides of CA (50, 51) showed that CyPA binds to loop 85-93 of HIV-1 CA, which exists in a random conformation in the native state and gains three-dimensional structure when bound to CyPA, as shown by the NMR and crystal structures of unligated CA and CA-CyPA (49, 79). Specifically, residues 85-93 of CA interacts with the CyPA active site residues of Arg55, Ile57, Phe60, Met61, Gln63, Asn71, Gly72, Thr73, Ala101, Asn102, Gln111, Phe113, Leu122, and His126 (Figure 6c). The side chain of Pro90 of CA occupied the hydrophobic pocket of CyPA, which is made up of residues of Phe60, Met61, Phe113, Leu122, and His126. Eight hydrogen bonds were formed between His87 Nd1 (CA) and Asn71 O (CyPA), Ala88 N and Gly72 O, Ala88 O and Gln63 NE2, Gly89 N and Asn102 O, Pro90 O and Arg55 NH1, Pro90 O and Arg55 NH2, Ile91 O and Trp121 NE1, Gln95 OE1 and Arg55 NH1 in the crystal structure of CyPA in complex with a fragment (residues 81-105) of CA (51). Overall, the CA fragment occupies the same binding pocket as a substrate peptide of PPIase and share many common interactions.

The role of CyPA in the HIV infectivity remains mystery. In consideration of the peptidyl-prolyl *cis-trans* isomerization is a rate determining step of protein folding, one might expect that CyP helps with the folding and thus assembly of CA into the core of the HIV-1 particle. However, the NMR and crystal structures of CA show the random conformation of the binding loop 85-93, implying that the Pro90 conformation is not critical for the folding of CA. This argument suggests that CyPA is unlikely to function as a *cis-trans* isomerase in HIV-1 infectivity. Very likely, the loop 85-93 in random conformation serves as a "knob" or "handle" to be grasped by other proteins such as CyP. As the consequence, the binding of CyP may trigger unfolding of the CA core and thus de-assemble of the HIV-1 particle. In the other words, CyP may act as a molecular chaperone, instead of PPIase, in the HIV-1 infectious process.

7. STRUCTURE OF CALCINEURIN-CYCLOPHILIN

7.1. Binding of CyPA-CsA to calcineurin

Calcineurin (CN) is a Ca^{2+}/calmodulin dependent serine/threonine protein phosphatase (PP2B) (84). The molecule of calcineurin is a heterodimer conprising a 59 kDa catalytic subunit calcineurin A (CNA) and a 19 kDa regulatory subunit calcineurin B (CNB). Calcineurin is involved in several biological processes, including immune system (84-88), cardiac hypertrophy (5-7), the second messenger cAMP pathway (87, 89), Na/K ion transportation in nephron (90), cell cycle regression in lower eukaryotes (91), and memory (92, 93). The most extensively studied function of CN is its involvement in the signal transduction pathway towards the T cell activation. Calcineurin is a common receptor for binding of two immunophilin-immunosuppressant families: CyP-CsA and FKBP-FK506 (2, 3, 94). The binding of CyP-CsA or FKBP-FK506 inhibits CN dephosphorylation activity on transcription factors such as nuclear factor of activated T cell (NFAT), thus leading to the suppression of T-cell

activation (4, 95). The immunophilin-immunosuppresant-CN-NFAT signaling pathway also plays critical roles in cardiac hypertrophy (96).

The catalytic subunit of human CNA-alpha (residues 1-521) contains a core phosphatase domain (residue 1-346), a CNB binding helix (BBH, residues 347-373), a calmodulin binding region (residues 390-414), and an autoinhibitory loop (residues 469-486) (Figure 7). The phosphatase domain consists of a central beta-sheet flanked by alpha-helices and has a folding similar to other serine/threonine protein phosphatases such as PP1 and PP2A (97). The BBH of CNA embeds in CNB and forms together with CNB a composite surface for binding of CyPA-CsA or FKBP-FK506. CNB comprises two domains, each of which has two calcium-binding sites with a folding similar to calmodulin. The conformations of CyPA, CNA, and CNB in the CyPA-CsA-CN complex (53, 54) are similar to the corresponding subunits in the CyPA-CsA binary complex (26, 30), unbound CN, and the FKBP-FK506-CN complex (98, 99).

The CsA residues 9-11 and 1-2 are involved in binding of CyPA in the ternary CyPA-CsA-CN complex and have similar conformation and interactions as that in the binary CyPA-CsA complex. The residues 3-8 of CsA bind to the composite surface made up of the residues from CNB and BBH of CNA. The recognition of CsA by CN is mainly achieved with two hydrogen bonds between Tyr341 OH of CNA and Ala7 N of CsA and between Try352 NE1 of CNA and Val5 O of CsA. Other CN residues involved in hydrophilic or hydrophobic interactions with CsA include Leu312, Val314, Tyr341, Trp342, Pro344, Trp352, Phe356 of CNA, and Met118, Val119, Asn112, and Leu123 of CNB. The backbone conformations of residues 3-8 of CsA are conserved in both binary and ternary complexes, but the side chain of MeLeu4 of CsA undergoes significant conformational changes upon binding of CyPA-CsA to CN.

Interactions between CN and CyPA involve residues from both the composite surface of CNA-CNB and the phosphatase domain of CNA (53, 54). Seven hydrogen bonds are observed: Arg122 (CNA)-Arg148 (CyPA), Glu359 (CNA)-Arg69 (CyPA), Glu47 (CNB) - Gly80 (CyPA), Gln50 (CNB) - Glu81 (CypA), Gln50 (CNB)-Lys82 (CyPA), Asn122 (CNB)-Ala103 (CyPA), and Lys164 (CNB)-Asn71 (CyPA). The residues involved in van der Waal interactions are: Arg122, Tyr159, Phe160, Leu312, Val314, Tyr315, Asn345, Pro355, Phe356, Glu359, and Thr362 of CNA, Glu47, Gln50, Asn122, Lys164 of CNB, and Pro58, Arg69, Asn71, Thr73, Glu81, Lys82, Ala103, Pro105, Trp121, Ser147, Arg148, and Asn149 of CyPA.

7.2. Common and distinct recognition of CyPA-CsA and FKBP-FK506 by CN

Two immunophilin-immunosuppresant complexes of CyPA-CsA and FKBP-FK506 bind to the same composite surface of CNA and CNB and share many of recognition elements (94). Among the 25 CN residues involved in hydrogen bonds or hydrophobic interactions with CyPA-CsA, twenty are common, but five (Arg122, Tyr315, Trp342, Thr362 of CNA, and Lys164 of CNB) uniquely interact with CyPA-CsA. Similarly, five residues of Asp313, Met347, Thr351 of CNA and Asn121, Gln127 of CNB contact only with FKBP-FK506. Four hydrogen bonds are commonly formed between CN and immunophilins: Trp352 of CNA and CsA or FK506, Glu47 of CNB with Gly80 of CyPA or Gln53 of FKBP12, Glu50 of CNB with Glu81 of CyPA or Glu54 of FKBP, and Asn121 of CNB with Ala103 of CyPA or Lys44 of FKBP12.

On the other hand, the recognition pattern in CN-CyPA-CsA is significantly different from CN-FKBP12-FK506. First, Tyr341 forms hydrogen bond with CsA, but van der Waals interaction with Arg42 of FKBP. Second, the majority of the hydrogen bonds between CN and CyPA-CsA are different from those with FKBP-FK506. Thus, five hydrogen bonds were uniquely observed in CN-CyPA-CsA: Arg122 (CNA)-Arg148 (CyPA), Tyr341 (CNA)-Ala7 (CsA), Glu359 (CNA)-Arg69 (CyPA), Gln50 (CNB)-Lys82 (CyPA), and Lys164 (CNB)-Asn71 (CyPA) (Figure 6). In comparison, four hydrogen bonds are unique for CN-FKBP-FK506: Tyr159 (CNA)-Asp32 (FKBP), Leu312 (CNA)-Lys35 (FKBP), Asn121 (CNB)-Lys44 (FKBP), and Gln127 (CNB)-Arg42 (FKBP) (98, 99). Particularly interesting is the hydrogen bond between Arg148 of CyPA and the active site residue Arg122 of CNA. This hydrogen bond may imply a direct regulation of the CN catalytic activity by the immunophilin, in addition to the special blockage.

The common but distinct recognition on CyPA-CsA and FKBP12-FK506 implies a capacity of the CN composite surface for binding of other inhibitory proteins such as the binding domain of AKAP79 (A-kinase anchoring protein 79), Cain (calcineurin inhibitory protein or Cabin), DSRC1 (Down's syndrome critical region) or MCIP (modulatory calcineurin interacting protein). AKAP79 was proposed to bind to the same recognition site as FKBP (99). On the other hand, the simultaneous interactions of CyPA-CsA with the composite surface and the active site as observed in the crystal structure may imply that the inhibitor binding surface serves as a general recognition site for binding of a protein substrate (53). Thus, a protein substrate must match the geometric shapes and chemical nature of the residues at both the immunophilin binding site and the active site of CN. Since BBH of CNA represents a unique C-terminal extension of the phosphatase domain among Ser/Thr protein phosphatases, the dual recognition may explain the narrow substrate specificity of CN.

8. PERSPECTIVES

Abundance of cyclophilins in human and other organisms implies its important roles in the biological systems. However, the *in vivo* function of CyP remains a mystery. Structural studies on CyPs in complex with the prolyl peptides and with capsid of HIV-1 imply that CyP is unlikely to serve as the *cis-trans* isomerase in the HIV-1 infectivity, instead, CyP may function as a molecular chaperone to assist with deassembly of HIV-1 particle.

While it is generally believed that CyP serves as a molecular chaperone in biological systems, the action modes and partnerships of CyP may vary in different systems. The structural study on the CyP40 complex with Hsp90 as well as a client protein will ultimately illustrate the function and action mechanism of CyP.

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