Crystal structure of the cyclophilin-like domain from the parasitic nematode *Brugia malayi*

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Abstract

Cyclophilins are a family of proteins that exhibit peptidyl-prolyl *cis-trans* isomerase activity and bind the immunosuppressive agent cyclosporin A (CsA). *Brugia malayi* is a filarial nematode parasite of humans, for which a cyclophilin-like domain was identified at the N-terminal of a protein containing 843 amino acid residues. There are two differences in sequence in the highly conserved CsA binding site: A histidine and a lysine replace a tryptophan and an alanine, respectively. The crystal structure of this domain has been determined by the molecular replacement method and refined to an *R*-factor of 16.9% at 2.15 Å resolution. The overall structure is similar to other cyclophilins; however, major differences occur in two loops. Comparison of the CsA binding site of this domain with members of the cyclophilin family shows significant structural differences, which can account for the reduced sensitivity of the *Brugia malayi* protein to inhibition by CsA.

Keywords: *Brugia malayi*; cyclophilin; nematode parasite; prolyl *cis-trans* isomerase; protein crystallography

Cyclophilins (Cyps) are ubiquitous and abundant proteins that exhibit peptidyl prolyl *cis-trans* isomerase (PPIase) activity in vitro (Fischer et al., 1989). PPIase catalyzes *cis-trans* isomerization about an Xaa-Pro peptide bond. The high degree of sequence conservation across phyla from bacteria to humans and the localization in various compartments within eukaryotic cells for the Cyps suggest that they have a function of fundamental importance for the cell cycle. The exact biological role of Cyp remains somewhat elusive, although it is thought that the Cyps facilitate correct folding and trafficking of proteins (Colley et al., 1991) and are likely to have chaperone activity (Ferreira et al., 1996). Cyp has also been found to be required for the infectious activity of HIV-1 virions (Braaten et al., 1996). Different classes of Cyps have been identified and were shown to bind the immunosuppressive drug cyclosporin A (CsA) to various degrees. CsA elicits most of its cellular effects by first binding to Cyp, forming a Cyp-CsA complex, which subsequently interacts with calcineurin and inhibits its ser-thr phosphatase activity (Schreiber & Crabtree, 1992).

The three-dimensional structure of a number of Cyps complexed with proline containing peptide (Kallen & Walkinshaw, 1992; Ke et al., 1993a; Konno et al., 1996) and CsA derivatives have been solved (Ke et al., 1993b; Thériault et al., 1993; Mikol et al., 1994), as have the structures of unliganded Cyps, by X-ray crystallogra-

phy and by NMR (Ke, 1992; Clubb et al., 1994). They share a common fold characterized by a β -barrel composed of eight anti-parallel β -strands flanked by two helices. Ligands are found to bind in an hydrophobic cleft at the surface of the barrel.

Brugia malayi is a filarial nematode parasite of humans found essentially in tropical Asia. The parasite resides in the lymphatic system and can cause elephantiasis in latter chronic stages. About 100 million people worldwide are estimated to be infected by filarial parasites. Interestingly, many parasite species including some filariae are killed by CsA (Chappell & Wastling, 1992; Page et al., 1995a). However, *Brugia* species are not susceptible to this effect (Lawrence & Denham, 1992; Page et al., 1995a).

A cDNA clone encoding a 843 amino acid protein from *B. malayi* was isolated and was shown to possess a N-terminal domain with sequence identities with Cyps ranging from 49 to 60% (Page et al., 1995a). This Cyp-like domain from *B. malayi* (CypBm-1) is most closely related to the larger Cyp-like proteins such as the Cyp from NK cells (Anderson et al., 1993) and the cyclophilin-40 protein (Kieffer et al., 1993). The PPIase activity of CypBm-1 exhibits a 45-fold reduced sensitivity to CsA inhibition when compared to human Cyps (Page et al., 1995a). There are two differences in sequence within the CsA binding site: a tryptophan and an alanine in CypHA are substituted for histidine and lysine, respectively, in CypBm-1. In this paper, we present the crystal structure of the CypBm-1 determined at 2.15 Å resolution, compare it with other known Cyp three-dimensional structures and provide a structural explanation for the reduced sensitivity of CypBm-1 to CsA inhibition.

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Results

Quality of the structure

The excellent quality of the electron density map allowed confident placement of all main- and side-chain atoms except the first two residues at the N-terminus, which were not observed in the density map. They were not included in the final model. Only 6 side chains out of 175 show noncontinuous electron density. These are: K3, K4, K55, K69, K80, and K161. None of these residues are within the CsA binding site. The statistics of the crystallographic refinement are shown in Table 1.

Overall structure

The structure of CypBm-1 shows the typical cyclophilin fold: a β -barrel composed of eight antiparallel β -strands closed off by two helices (Fig. 1). There is no noticeable differences in elements of secondary structure when compared with other members of the Cyp family (Fig. 2). The RMS deviation (RMSD) between the CypBm-1 C α backbone atoms within the elements of secondary structure with those of CypHA (Ke, 1992), CypHB (Mikol et al., 1994), and CypcEc (Konno et al., 1996) are 0.8, 0.7, and 1.2 Å, respectively (Fig. 3). The main differences occur for two loops (1) the CD loop, which contains an eight amino acid insert sequence when compared with CypHA, and (2) the JK loop, which delineates one of the rim of the CsA binding cleft. The CD loop in CypBm-1 connects the first helix α C to the third strand β D and it consists of 19 amino acid residues. This loop protrudes out toward the solvent and does not make any crystal packing interactions. The CD loop is composed of three successive β -turns: a type IV turn for residues 45–48, a type I turn for residues 51–54, and a type II turn for residues 59–62. The amino acid insert adopts a rather stable conformation as indicated by the averaged temperature factor value for all atoms of the nine residues 48:56 (14.6 Å²). This loop lies more than 15 Å away from the CsA binding site. The 13 amino acid JK loop (Y155-D167), which connects the second α -helix to the last β -strand adopts in CypBm-1 a very similar conformation to those of CypBH (RMSD of 0.4 Å for the 13 main

Fig. 1. Schematic representation of CypBm-1. All pictures representing molecules were prepared with the program MOLSCRIPT (Kraulis, 1991).

chain atoms) and of murine CypC (0.4 Å), but significantly different than the 12 amino acid loop (R144-K155) in CypHA (RMSD of 3.4 Å).

CsA binding site

Thirteen residues from CypHA R55, F60, M61, Q63, G72, A101, N102, A103, Q111, F113, W121, L122, and H126 are located within 4.0 Å from CsA in the CypHA/CsA complex and define the CsA binding site (Mikol et al., 1993). Two residues out of the 13

Table 1. Crystallographic refinement statistics

Resolution limits (Å)	100.0–2.15
R-factor ^a	0.169
R-free (5% of data)	0.211
No. of cyclophilin non-H atoms	1,338
No. of solvent molecules	234
Ramachandran plot	
% of residues in the most favorable region	83.4
% of residues in the additional allowed region	16.6
% of nonglycine residues in disallowed regions	0.0
B-factors (Å ²)	
Protein atoms	12.0
Solvent atoms	27.9
Weighted RMSDs from ideality	
Bond length (Å)	0.012
Bond angle (°)	1.60

^a.R-factor = $\sum ||F_o| - |F_c|| / \sum |F_o|$, where $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively.

Fig. 2. Sequence alignment of CypBm-1, human cyclophilin A (CypHA), human cyclophilin B (CypHB), cytoplasmic *Escherichia coli* cyclophilin (CypcEc) and cyclophilin from NK cells (CypHNK). The sequence identities between CypBm-1 and CypHA, CypHB, CypcEc, and CypHNK are 52%, 57%, 26%, and 60%, respectively. The secondary structural elements from CypBm-1, CypHA, CypHB, and CypcEc as deduced from their crystal structures are underlined. The prefixes α , β , 3_{10} and β designate α -helix, 3_{10} helix and β -strand, respectively. Dots represent identical residues with CypBm-1 and dashes insertions. Stars indicate residues that are within 4 Å from the CsA as observed in the CypHA/CsA complex (Mikol et al., 1993). The sequential notation for CypBm-1 is used.

Fig. 3. Stereopicture of the overlay of CypBm-1 on CypHA(a) and on CypHB(b). The C α trace of CypBm-1 (continuous lines) is superimposed on that of CypHA (broken lines) (A) and on that of CypHB (broken lines) (B). Some residues of CypBm-1 are labeled.

residues from the CsA binding site in CypHA are not conserved in CypBm-1. These include A103 and W121 from CypHA, which are substituted by K114 and H132 in CypBm-1. The RMSD for all atoms of the 11 conserved residues of the CsA binding site between CypHA and CypBm-1 is 1.0 Å indicative of an overall conserved binding site. However, there are significant structural differences for F71 and around the two nonconserved residues K114 and H132 (Fig. 4). The side chain of R66 also adopts a different conformation than in the CypHA/CsA complex presumably because of crystal packing. The N $^{\epsilon}$ from K114 (A103 in CypHA) points toward the EF loop and sits 0.7 Å away from the position observed for the C α of the amino butyric acid from CsA in the CypHA/CsA complex. It is engaged in h-bond with the carbonyl oxygen of G83 (distance 2.8 Å), thus limiting the access to the amino butyric acid. The average *B*-factor for K114 is 12.9 Å². The small 3_{10} helix containing H132 has moved away

from the CsA binding site toward the EF loop by 1.4 Å when compared with CypHA. Consequently, the imidazole ring of H132 does not occupy the position of the five-membered ring of the indole group from W121 in the CypHA/CsA complex (Fig. 4). However, it must be mentioned that the side chain of H132 is involved in crystal packing interaction. The side chain of F71 adopts a different conformation than the F60 in CypHA. The phenyl ring is moved toward the interior of the barrel probably as a result of the void created by the absence of γ -substitution on the side chain of residue 130 (Ala in CypBm-1, Thr in CypHA).

Discussion

The eight amino acid insert from the CD loop was speculated to be responsible for a conserved function such as a binding or recognition site (Kieffer et al., 1993). A three-dimensional search with

Fig. 4. CsA binding site. **A:** Simulated annealed omit $(2|F_{obs}| - |F_{calc}|)e^{i\phi_c}$ map of the CsA binding site in CypBm-1 contoured at 1σ and computed for data between 100.0 and 2.15 Å. All atoms in CypBm-1 that are found within 3.5 Å of CsA after superimposition of the CypHA/CsA complex were omitted. Carbon, oxygen, and nitrogen atoms are represented in yellow, red, and blue, respectively. **B:** Comparison of the CsA binding site in CypBm-1 (purple lines) and in CypHA (green lines). The 13 residues defining the CsA binding are shown and CsA as observed in the crystal structure of CypHA/CsA complex (Mikol et al., 1993; PDB code 1CWA). Residue A130 is also represented. CsA is displayed as a ball-and-stick model with black bonds, carbon, oxygen, and nitrogen atoms are represented in yellow, red, and blue, respectively. Residue numbers are given for CsA and labels are displayed for the CypBm-1 residues. The hydrogen bond between the N^ε from K114 and G83 is represented with broken lines.

the PDB shows high structural similarity between the 11 amino acids G48–H48 from CypBm-1 and the residues T242–W252 from the Cys2 activator binding domain of protein kinase C δ (pdb code 1PTR) with an RMSD of 0.25 Å for the 11 C α s (Fig. 5, Table 2). The main chain atoms of G50–G54 and the carbonyl oxygen of T49 from CypBm-1 protrude out toward the solvent, whereas the side-chain atoms face the rest of the protein molecule. It is also observed for the corresponding loop in 1PTR. This five residues segment might provide a template for protein/Cyp recognition. Although the conformational homology with 1PTR might be purely fortuitous and only structural, it is striking to notice that the protein kinase C is involved in the recognition of a great number of protein substrates.

Functional analysis of CypBm-1 demonstrated that the PPIase activity is similar ($k_{cat}/K_m = 8 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$) to that of CypHA ($k_{cat}/K_m = 1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$). It is consistent with the fact that the active site residues involved in the PPIase activity are conserved in sequence and in three-dimensional structure except F71, which is displaced.

The reduced sensitivity to CsA inhibition could be accounted for by the three structural differences in the CsA binding site between CypBm-1 and CypHA. (1) The side chain of K114 partly occupies the position of the aminobutyric acid from CsA. Binding of CsA would require flipping of the side chain. (2) H132 does not sit at the correct position to be engaged in h-bond with the carbonyl oxygen of N-methylLeucine9 of CsA, neither does it provide the hydrophobic surface for Van der Waals contact. These interactions observed for W121 in CypHA were found to be critical for tight binding (Liu et al., 1991). (3) The F71 ring adopts a conformation that renders the binding cleft more shallow.

It has been shown that CsA displays a broad spectrum of anti-parasitic effects (Chappell & Wastling, 1992). The mechanism of action has not yet been unraveled; however, it is likely to involve inhibition of the PPIase activity or interference with a critical signal transduction pathway. *B. malayi* is not killed by CsA even at high doses and is now known to possess two Cyps with PPIase activities: an insensitive-CsA form, CypBm-1, which contains two mutations in the CsA binding site with respect to CypHA (W132H and A114K), and a sensitive form, which shows sequence identity with CypHA in the CsA binding site (Ma et al., 1996). Among the

CsA-sensitive parasites only Trp-containing Cyp have been found so far (Page et al., 1995b). It suggests that the second Cyp identified from *B. malayi* is not important and that CypBm-1 might confer resistance to CsA. None of the known human cyclophilins identified so far possess a similar CsA binding site as CypBm-1 thus qualifying CypBm-1 as a potential drug target. The knowledge of the three-dimensional structure of CypBm-1 and the yet known structural differences between CypHA and CypBm-1 could aid in the discovery of specific drugs for the treatment of important infectious diseases.

Materials and methods

Preparation of the crystals

CypBm-1 was expressed and purified as previously described (Page et al., 1995a) with some modifications: Plasmid pMal-c2 (New England Biolabs, Massachusetts) was digested with XmnI and HindIII. The sequence of the forward PCR primer corresponded to the open reading frame of Bmcp-1 was 5'-ATGTCAAAAAAAG ATCGCCGCCGG-3'. The PCR product was digested with HindIII, and then ligated into the linearized pMal-c2. The purified CypBm-1 was concentrated to 14 mg/ml. Crystals of CypBm-1 were grown by vapor diffusion at 19 °C by the hanging drop method. The precipitating solution in the well consisted of 100 mM MES-NaOH (pH 6.0), 1.8 M (NH₄)₂SO₄. The initial 6 μ L drop consisted of 50 mM MES-NaOH (pH 6.0), 0.9 M (NH₄)₂SO₄, 1 mM CypBm-1. Large single crystals of about 0.4 mm $\times$ 0.3 mm $\times$ 0.1 mm appeared within a week.

Data collection and processing

A crystal was placed into a 10 μ L drop of a solution containing 100 mM MES-NaOH pH 6.0, 56% PEG₄₀₀ for 2 min. The crystal was picked up with a fiber loop and flash cooled in a stream of gaseous nitrogen at 95 K. The X-ray intensity data were collected on DIP2000 imaging plate (Mac Sciences, Japan) mounted on a FR591 rotating anode (Nonius, The Netherlands) operated at 50 kV 90 mA. Refined cell dimensions are $a = b = 57.9 \text{ Å}$, $c =$

Fig. 5. Comparison of the 11 residues from the CD loop between CypBm-1 (red lines, sequence GTGKIGKPLH) and the Cys2 domain from PKC (green lines, sequence TFCDHGSLW). The C α traces of CypBm-1 and of the protein kinase C fragment are also shown. Some labels for CypBm-1 residues are displayed.

Table 2. 3D similarity between the CD loop from CypBm-1 and known protein structures from the PDB^a

Reference segment	Number of residues	RMS (Å)	Match segment	Name of protein	PDB code
G48-H58	11	0.25	242–252	Cys2 domain of PKC	IPTQ
G48-P56	9	0.2	24–32	DNA-binding protein	1RMD
G48-P56	9	0.2	88–96	Alkaline phosphatase	1ALJ
G48-P56	9	0.3	A240–A248	CytochromeP450 BM-3	1FAH
G48-P56	9	0.3	B144–B152	Adenylate kinase	1AKE
G48-P56	9	0.3	752–760	Aldehyde oxidoreductase	1ALO
G48-P56	9	0.3	H101–H109	Methylamine dehydrogenase	1MAE
G48-P56	9	0.3	136–144	Heterogeneous nuclear ribonucleoprotein	1HAI
G48-P56	9	0.3	A341–A349	Biotin carboxylase	1BNC

^aThe search for structural similarity of the CD loop with other known loop structures was performed within all protein X-ray structures deposited with the PDB using the ESCAN program (Escalier et al., 1998). The query fragment was composed of the 12 C α 's from the amino acid residues M46–H58. A RMS of 0.25 Å for 11 atoms corresponds to 4 standard deviations from the mean.

140.13 Å, $\alpha = \beta = \gamma = 90^\circ$ and the space group is P4₁2₁2. The crystal contains one molecule per asymmetric unit and 54.1% solvent ($V_M = 2.67 \text{ Å}^3/\text{Da}$). A total of 84,704 reflections were measured, which resulted in 13,361 unique reflections to a 2.15 Å d spacing ($R_{\text{sym}} = 5.0\%$). The completeness of the data is 98.6% between 10.0 and 2.15 Å and 96% for data between 2.23 and 2.15 Å. The R_{sym} is 8.0% in the last resolution. Data processing and scaling were carried out using DENZO and SCALEPACK (Otwinowski & Minor, 1997).

Structure solution and crystallographic refinement

The structure was solved by molecular replacement using a search model made from the coordinates of CypHA (Mikol et al., 1993), where all residues that differ from CypBm-1 in sequence in the presumed elements of secondary structure were set to alanine and residues in the predicted loops were removed. The correct space group (P4₁2₁2) was confirmed using translation function calculations. At this stage, inspection of a $(F_{\text{obs}} - F_{\text{calc}})e^{i\phi_c}$ difference Fourier map showed some clear density in terms of changes for most of the sequence of CypBm-1 relative to CypHA and for residues of the loops. The sequence of the model was changed to that corresponding to CypBm-1 in alternating cycles of manual rebuilding using O (Jones et al., 1991) and least-squares refinement using X-PLOR (Brünger, 1992). The final R -factor is 16.9% and details of the refinement statistics are given in Table 1. Atomic coordinates and structure factors have been deposited with the PDB (accession code 1a33).

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References

- Anderson SK, Gallinger S, Roder J, Frey J, Young HA, Ortaldo JR. 1993. A cyclophilin-related protein involved in the function of natural killer cells. *Proc Natl Acad Sci USA* 90:542–546.
- Braaten D, Franke EK, Luban J. 1996. Cyclophilin A is required for the replication of group M human immunodeficiency virus type 1 (HIV-1) and

- simian immunodeficiency virus SIVcpzGAB but not group O HIV-1 or other primate immunodeficiency viruses. *J Virol* 70:4220–4227.
- Brünger AT. 1992. *X-PLOR version 3.1: A system for X-ray crystallography and NMR*. New Haven: Yale University Press.
- Chappell LH, Wastling JM. 1992. Cyclosporin A: Antiparasitic drug, modulator of the host parasite relationship and immunosuppressant. *Parasitology* 105: S25–S40.
- Clubb RT, Ferguson SB, Walsh CT, Wagner G. 1994. Three-dimensional solution structure of *Escherichia coli* periplasmic cyclophilin. *Biochemistry* 33:4093–4100.
- Colley NJ, Baker EK, Stamnes MA, Zuker CS. 1991. The cyclophilin homolog ninaA is required in the secretory pathway. *Cell* 67:255–263.
- Escalier V, Pothier J, Soldano H, Viari A. 1998. Pairwise and multiple identification of three-dimensional common substructures in proteins. *J Comput Biol*. In press.
- Ferreira PA, Nakayama TA, Pak WL, Travis GH. 1996. Cyclophilin related protein RanBP2 acts as chaperone for red/green opsin. *Nature* 383:637–640.
- Fischer G, Wittmann-Liebold B, Lang K, Kiefhaber K, Schmid FX. 1989. Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature* 337:476–478.
- Jones AT, Zou J-Y, Cowan SW, Kjeldgaard M. 1991. Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr sect A* 47:110–119.
- Kallen J, Walkinshaw MD. 1992. The X-ray structure of a tetrapeptide bound to the active site of human cyclophilin A. *FEBS Lett* 300:286–290.
- Ke H. 1992. Similarities and differences between cyclophilin A and other β -barrel structures. *J Mol Biol* 228:539–550.
- Ke H, Mayrose D, Cao W. 1993a. Crystal structure of cyclophilin A complexed with substrate Ala-Pro suggests a solvent assisted mechanism of cis-trans isomerization. *Proc Natl Acad Sci USA* 90:3324–3328.
- Ke H, Zhao Y, Luo F, Weissman I, Friedmann J. 1993b. Crystal structure of murine cyclophilin C complexed with immunosuppressive drug cyclosporin A. *Proc Natl Acad Sci USA* 90:11850–11854.
- Kieffer JL, Seng TW, Li W, Osterman DG, Handschumacher RE, Bayney RM. 1993. Cyclophilin-40, a protein with homology to the P59 component of the steroid receptor complex. *J Biol Chem* 267:5503–5507.
- Konno M, Ito M, Hayano T, Takahashi N. 1996. The substrate-binding site in *Escherichia coli* cyclophilin A preferably recognizes a cis-proline isomer or a highly distorted form of the trans isomer. *J Mol Biol* 256:897–908.
- Kraulis P. 1991. MOLSCRIPT: A program to produce both detailed and schematic plots of protein structures. *J Appl Cryst* 24:946–950.
- Lawrence R, Denham DA. 1992. *Brugia pahangi* infections in immune-compromised rats demonstrate that separate mechanisms control adult worm and microfilarial numbers. *Parasite Immunol* 14:371–384.
- Liu J, Chen CM, Walsh CT. 1991. Human and *Escherichia coli* cyclophilins: Sensitivity to inhibition by the immunosuppressant cyclosporin A correlates with a specific tryptophan residue. *Biochemistry* 30:2306–2310.
- Ma D, Hong X, Raghavan N, Scott AL, McCarthy JS, Nutman TB, Williams SA, Carlow CKS. 1996. A cyclosporin A-sensitive small molecular weight cyclophilin of filarial parasites. *Mol Biochem Parasitol* 79:235–241.
- Mikol V, Kallen J, Walkinshaw M D. 1993. X-ray structure of a monomeric cyclophilin A-cyclosporin A crystal complex at 2.1 Å resolution. *J Mol Biol* 234:1119–1130.

- Mikol V, Kallen J, Walkinshaw MD. 1994. X-ray structure of a cyclophilin B/cyclosporin complex: Comparison with cyclophilin A and delineation of its calcineurin-binding domain. *Proc Natl Acad Sci USA* 91:5183–5186.
- Page AP, Kumar S, Carlow CKS. 1995b. Parasite cyclophilins and antiparasite activity of cyclosporin A. *Parasitology Today* 11:385–388.
- Page AP, Landry D, Wilson GG, Carlow CKS. 1995a. Molecular characterisation of a cyclosporin A-insensitive cyclophilin from the parasitic nematode *Brugia malayi*. *Biochemistry* 34:11545–11550.
- Otwinowski Z, Minor W. 1997. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol* 276:307–326.
- Schreiber SL, Crabtree GR. 1992. The mechanism of action of cyclosporin A and FK506. *Immunol Today* 13:136–142.
- Thériault Y, Logan TM, Meadows R, Yu L, Olejniczak ET, Holzman TF, Simmer RL, Fesik SW. 1993. Solution structure of the cyclosporin A/cyclophilin complex by NMR. *Nature* 361:88–91.