The X-ray structure of a divergent cyclophilin from the nematode parasite *Brugia malayi*

Paul Taylor[a], Antony P. Page[b], George Kontopidis[a], Holger Husi[a], Malcolm D. Walkinshaw[a,*]

[a] *Structural Biochemistry Group, The University of Edinburgh, Michael Swann Building, King's Buildings, Edinburgh EH9 3JR, UK*
[b] *Wellcome Unit of Molecular Parasitology, The Anderson College, 56 Dumbarton Road, Glasgow University, Glasgow G11 6NU, UK*

Received 22 January 1998; revised version received 24 February 1998

Abstract A structure of residues 1–177 of the cyclophilin domain of a large divergent cyclophilin from the filarial nematode parasite *Brugia malayi* has been crystallised and solved in two different crystal forms. The active site has a similar structure to that of human cyclophilin A. Two of the 13 residues important in forming the human cyclophilin A/cyclosporin A complex are altered in the *B. malayi* cyclophilin and explain the relatively poor inhibition of peptidyl prolyl isomerase activity by cyclosporin A.

1. Introduction

Cyclophilins are ubiquitous proteins, found in organisms as diverse as plants, bacteria and mammals [1]. Many distinct isoforms have been found in a single species, with 11 separate isoforms being described in the free-living nematode *Caenorhabditis elegans* [2]. These proteins possess peptidyl prolyl isomerase (PPIase) activity, and act as catalysts in many protein folding events, especially in the folding of proline-rich proteins [3,4]. The cyclophilins also characteristically bind the immunosuppressive drug cyclosporin A (CsA) [5], and the resulting complex with the cellular protein phosphatase calcineurin specifically inhibits signal transduction events in mammalian T-cells [6]. Independent of its immunosuppressive activity CsA has powerful killing properties against a wide range of infectious organisms including large multicellular helminth parasites [7]. The precise mode of action of CsA, or its derivatives, against protozoan and helminth parasites is presently unknown and may involve an interruption of an essential signal transduction pathway, or inhibition of PPIase activity resulting in improper protein folding of a vital parasite molecule(s). Interestingly, this compound characteristically causes structural, tegumental damage to a wide range of helminths [7,8] and may be affecting the latter.

X-ray and NMR structures of a number of cyclophilins have been studied [9]. Sequence and structural comparisons of the cyclophilins allow grouping of different isoforms depending on their cellular location, and their degree of amino acid sequence conservation. The most abundant form, cyclophilin A, is found in the cytosol. Cyclophilin B is located in the endoplasmic reticulum [10] and another isoform is located in the mitochondrion [11]. There is also a growing family of divergent cyclophilins which are not highly conserved and have secondary non-cyclophilin domains. The cyclophilin-40 family [12] for example has a carboxy-terminal domain required for binding to the estrogen receptor [13]. There are also a number of examples where these domains are either located amino, carboxyl or in some instances flank the cyclophilin domains. While detailed structural information exists for the highly conserved cyclophilins [9], very little is known about these divergent isoforms.

Cyclophilins have now been found in numerous parasites, including the human filarial nematode *Brugia malayi* [14]. *B. malayi* is a medically important parasite found in tropical countries, and infection is transmitted via mosquito vectors and can result in chronic debilitating filarial disease symptoms, which can include elephantiasis. Together with the other major filarial species, *B. malayi* currently infects 100 million people worldwide, and over 900 million people are at risk from infection from *Brugia* and related filarial species [15]. The divergent cyclophilin isoform in this species (bmCyp-1) has been characterised biochemically and has been shown to posses PPIase activity which is relatively insensitive to CsA, a fact consistent with an altered CsA-binding domain [14].

In this paper we describe the X-ray structure of this divergent nematode cyclophilin which can be used to explain its relative insensitivity to cyclosporin A.

2. Material and methods

2.1. Preparation and purification of recombinant *B. malayi* cyclophilin

Primers were designed corresponding to the amino-terminal cyclophilin domain of *bmcyp-1* (Genbank L37292). The forward primer corresponded to the open reading frame of *bmcyp-1* with the addition of an upstream *Bam*HI recognition site (underlined) and had the sequence 5′ GGGGATCCATGTCAAAAAAAGATCGCCG 3′. The reverse primer corresponded to the 3′ end of the cyclophilin domain (including amino acid 177), and contained a downstream termination codon and a *Hin*dIII recognition site (underlined) and had the sequence 5′ CGGAAGCTTCAAACAAGTTCACCACAATTAAGTAT 3′. These primers were used in the following thermal cycling reaction: 10 pmol of each primer, 1×buffer (45 mM Tris pH 8.8; 11 mM ammonium sulphate; 4.5 mM $MgCl_2$; 6.7 mM 2-ME; 4.4 μM EDTA (pH 8); 1 mM of each dNTP; 113 μg/ml non-acetylated BSA), 1 μl of an adult male λgt11 cDNA library (a kind gift from Dr. Mario Philipp, Tulane Primate Center, LA, USA) and 1 μl Taq polymerase (Advanced Biotechnologies), cycled 20× at 94°C for 30 s, 58°C for 1 min and 72°C for 1 min in 100 μl. The resultant product was phenol/chloroform extracted, ethanol precipitated, restriction digested with *Bam*HI and *Hin*dIII and gel purified from a 1% agarose gel using a QIAquick gel extraction kit (Qiagen). This product was ligated into similarly digested and purified plasmid pMAL-c2 (New England Biolabs), generating a fusion protein with maltose-binding protein (MBP). Plasmid DNA was isolated (Qiagen kit) and the insert was sequenced in both directions, establishing that no mutations had been generated during the PCR reaction. The protocol used for the production and purification of the MBP-fusion protein was as outlined in the MBP-fusion protein manual (New England Biolabs). Expression of the plasmid in XL-1 blue supercompetant cells (Stratagene) pro-

*Corresponding author.

PII S0014-5793(98)00264-6

duced 23 mg/l of amylose-purified fusion protein. Cleavage of MBP from the fusion protein was performed at 4°C for 48 h with 1% (w/w) factor Xa protease (New England Biolabs). The recombinant cyclophilin (177 aa) with a 6 amino acid in-frame N-terminal fusion derived from the vector multiple cloning site (Ile-Ser-Glu-Phe-Gly-Ser) was separated from the MBP by fast protein liquid chromatography (FPLC) using a Mono-Q anion exchange resin (Pharmacia). The homogenous sample was then dialysed overnight against 20 mM Tris (pH 7.4) in the absence of salts and concentrated to 8.6 mg/ml in a Centricon 10 concentrator (Amicon).

Two crystal forms of bmCyp-1 were grown from the 183 aa long protein isolated above. Both forms were grown using the hanging drop method.

2.1.1. Trigonal form. Trigonal form of bmCyp-1: The 4 μl hanging drop consisted of 10 mM Tris/HCl at pH 7.4, 6.5% w/v PEG 4000, 50 mM ammonium acetate, 0.02% w/v NaN_3, 0.4 mM bmCyp-1. The drop was suspended over a well containing 20 mM Tris/HCl pH 7.4, 13% w/v PEG 4000, 100 mM ammonium acetate, 0.02% w/v NaN_3. The crystals are trigonal with $a = b = 88.9$ Å and $c = 106.4$ Å and space group $P3_12_12$. Crystals were dipped in a cryo-solution of 26% glycerol, 13% PEG 4000, 100 mM ammonium acetate, 0.02% w/v NaN_3 and flash frozen in liquid nitrogen. Data collection was carried out at 100 K (Oxford cryosystems) using a MAR 300 image plate on the Daresbury SRS using a wavelength of 1.488 Å. 206 863 data were collected giving 38 564 unique data to 1.9 Å with a completeness of 99.7% and an $R_{merge} = 6.3\%$. The data were processed using DENZO [16]. The structure was solved using the molecular replacement protocol from AMORE [17] with the search model consisting of CypA from the CypA/CsA complex [18]. Only one peak was found in the rotation function. The translation function showed a solution consistent with two molecules in the asymmetric unit related by an almost exact translation of $c/2$. $V_m = 3.02$ Da/Å^3 for 12 molecules in the unit cell.

The structure was refined using alternating rounds of positional and *B* factor refinement using the data in the resolution range 5–1.9 Å, with ordered water molecules added to the refinement as they became apparent in difference Fourier maps. 10% of the data were randomly selected to form a test data set to be used to calculate R_{free}.

All loop insertions and deletions were modelled into electron density maps for both molecules, however refinement stuck at $R = 32\%$. The packing arrangement suggested that the crystal may be composed of an intimate twin. It was possible to refine the structure using half the data in a unit cell with dimensions $a = b = 88.9$ Å, $c = 53.2$ Å, and space group $P3_22_12$. This suggested that one possible twin may be a

```
                                                                  $
bmCyp1   (1)   MSKKDRRRVF LDVTIDGNLA GRIVMELYND IAPRTCNNFL MLCTGMAGTG   (50)
ceCyp8   (3)   PEVRG.K.A. F.IS.N.EP. ....FS.W.H CC...VE..R AF...--EL.   (50)
hnkCyp  (55)   .GAQ..PQCH F.IE.NREPV ...MFQ.FS. .C.K..K... C..S.EK.L.   (104)
 srCyp   (2)   GI.VQ.P.C. F.IA.NNQP. ..V.F..FS. VC.K..E..R C....EK...   (51)
hCyp40  (10)   P.NPSNP... F..D.G.ERV ....L..FA. .V.K.AE..R A....EK.I.   (59)
 hCypB   (6)   KGP.VTVK.Y F.LR.GDEDV ..VIFG.FGK TV.K.VD..V A.A..EK.F.   (55)
 hCypA   (1)   ~~~MV.PT.. F.IAV..EPL ..VSF..FA. KV.K.AE..R A.S..EK.F.   (47)

                                *     ** *        *
bmCyp1  (51)   KISGKPLHYK GSTFHRVIKN FMIQGGDFTK GDGTGGESIY GGMFDDEEFV   (100)
ceCyp8  (51)   .MN.HYAS.Q ..V......G .......I.H .N....Y... .RT....NLA   (100)
hnkCyp(105)   .TT..K.C.. .......V.. ........SE .N.K...... ..Y.K..N.I   (154)
 srCyp  (52)   .STQ...... SCL....V.D ..V.....SE .N.R...... ..F.E..S.A   (101)
hCyp40  (60)   HTT.....F. .CP...I..K ........SN QN........ .EK.E..N.H   (109)
 hCypB  (56)   --------.. N.K......D .........R ......K... .ER.P..N.K   (97)
 hCypA  (48)   --------.. ..C...I.PG ..C......R HN....K... .EK.E..N.I   (89)

                           ***          * *         **    *
bmCyp1(101)   MKHDEPFVVS MANKGPNTNG SQFFITTTPA PHLNNIHVVF GKVVSGQEVV   (150)
ceCYP8(101)   L..KK.YLL. ...R..D... ......SEEV ...DGK.C.. .E.IK.V...   (150)
hnkCyp(155)   L...RA.LL. ...R.KH... .......K.. ...DGV.... .L.I..F..I   (204)
 srCyp(102)   V..NKE.LL. ...R.KD... .......K.T ...DGH.... .Q.I......   (151)
hCyp40(110)   Y...RAGLL. ...A.R.... .......V.T ...DGK.... .Q.IK.IG.A   (159)
 hCypB (98)   L..YG.GW.. ...A.KD... .......VKT AW.DGK.... ...LE.M...   (147)
 hCypA (90)   L..TG.GIL. ...A...... .....C.AKT EW.DGK.... ...KE.MNI.   (139)

                                          $
bmCyp1(151)   TKIEYLKTNS KNRPLADVVI LNCGELV    (177)  [100%]
ceCyp8(151)   KA..N.E.GN EDK.VCK.E. TH...M.    (177)   [54%]
hnkCyp(205)   EQ..N...DA AS..Y...RV ID..V.A    (231)   [60%]
 srCyp(152)   RE..NQ..DA ASK.F.E.R. .S....I    (178)   [59%]
hCyp40(160)   RIL.NVEVKG E-K.AKLC.. AE.....    (185)   [56%]
 hCypB(148)   R.V.ST..D. RDK..K..I. AD..KIE    (174)   [54%]
 hCypA(140)   EAM.RFGSRN G-KTSKKIT. AD..Q.E    (165)   [49%]
```

Fig. 1. Amino acid comparison of cyclophilin domains with divergent *B. malayi* cyclophilin. Sequences analysed by GCG pile-up, dashes introduced for maximum alignment. bmCyp-1, *B. malayi* Cyp-1 isoform (L37292); ceCyp-8, *C. elegans* Cyp-8 isoform (U31078); srCyp, human divergent S/R-rich cyclophilin (X99717); hnkCyp, human natural killer cell cyclophilin (L04288); hCyp-40, human cyclophilin-40 isoform (L11667); hCypB, human cyclophilin B isoform (M60857); hCypA, human cyclophilin A isoform (X52851). Dots represent residues identical to bmCyp-1. $ indicates highly conserved cysteines in the divergent cyclophilins. * represents the highly conserved residues which make contact with cyclosporin A, and altered residues in the divergent Cyps are indicated in bold. Italics represent the eight amino acid extension common to the divergent cyclophilins. Parenthesis indicates the percentage identity to bmCyp-1. The bmCyp-1 protein used in crystallographic studied was preceded with the following six amino acid sequence from the cloning vector, Ile-Ser-Glu-Phe-Gly-Ser.

mixture of this small unit cell with one molecule per asymmetric unit overlapped with a larger cell with two molecules per asymmetric unit. No further attempt was made to define the trigonal structure or to model the possible twinning and all further descriptions of molecular structure are based on the tetragonal crystal form. It is however worth noting that the inserted loop (51-KISGKPLH-58) adopts the same conformation in the trigonal structure and the tetragonal structure (described below) and is therefore likely to be independent of crystal packing effects.

2.1.2. Tetragonal form. The 3 μl hanging drop consisted of 50 mM imidazole at pH 6.5, 15% saturated ammonium sulphate, 0.02% w/v NaN_3, 0.56 mM bmCyp-1. The drop was suspended over a well containing 100 mM imidazole at pH 6.5, 30% saturated ammonium sulphate and 0.02% w/v NaN_3. The crystals are tetragonal with $a=b=57.2$ Å and $c=141.9$ Å and space group $P4_12_12$. One crystal was dipped in a cryo-solution of 27% glycerol, 100 mM imidazole at pH 6.5 and 30% saturated ammonium sulphate and flash frozen in liquid nitrogen. Data collection at 100 K (Oxford cryosystems) using a MAR 300 image plate using Cu Kα radiation from a rotating anode source. 37 494 data were measured to give 6173 unique data, with an R_{merge} of 10.2% and 95.2% completeness to a resolution of 2.8 Å. These data were sufficient to solve the structure using AMORE [17] which gave one clear peak in the rotation function and a clear translation function solution. Using the same crystal, data were collected on station 7.2 at the Daresbury SRS with a wavelength of 1.488 Å. A total of 103 228 data were collected which gave 18 055 unique data (96.9% complete to 1.95 Å) with $R_{merge}=9.2\%$. The structure was initially refined using SHELX97 [19] with 10% of the data randomly selected to calculate R_{free}. Further refinement was carried out using XPLOR [20] with the same data set. Some eight residues at the N-terminus could not be located in the electron density. Refinement was carried out using alternate rounds of positional and *B* factor refinement using all data in the resolution range 8–1.95 Å. A total of 155 ordered water molecules were added to the refinement as they became apparent in difference Fourier maps. At this point a bulk solvent correction was applied [21] with a density level of 0.34 $e\text{Å}^{-3}$ with a *B* value of 49 Å^2. The final *R* factor was 19.9% with $R_{free}=23.4\%$. RMS deviation from ideality in bond length, bond angle and torsion angle are 0.008 Å, 1.426° and 26.02° respectively. The mean temperature factor (*B*) for protein atoms is 27.2 Å^2 and for water molecules the average *B* value is 43 Å^2. A Ramachandran plot showed all of the non-glycine residues were in the most favoured regions.

Atomic coordinates have been deposited in the Brookhaven Database with the code 1A58.

3. Results and discussion

The complete gene for bmCyp-1 encodes an 843 aa two domain protein, having an N-terminal cyclophilin domain of 177 amino acids, followed by a highly charged, hydrophilic carboxy-terminal domain of 666 amino acids [14]. In Fig. 1 the cyclophilin domain of this protein is aligned with the closest homologues from man and the free-living nematode *C. elegans*, and also with the structurally well characterised human cytosolic isoform CypA and the secreted isoform CypB. The degree of homology is significantly high between the *C. elegans* Cyp-8, the large divergent human natural killer cell and SR- cyclophilin isoforms, and this homology also extends into the preceding C-terminal domains (not shown). The Cyp domains of these four divergent cyclophilin proteins are between 54 and 60% identical. Sequence identity drops to 49% between bmCyp-1 and the hCypA isoform.

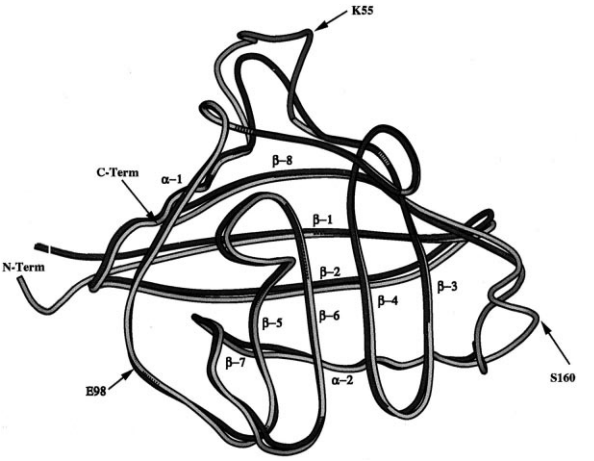


Fig. 2. Overlay of bmCyp-1 (pale grey) with hCypA (dark grey) from a least squares fit using corresponding backbone atoms. The octapeptide insert (51-KISGKPLH-58) in bmCyp-1 is highlighted. The eight β-strands and two longer α-helices are labelled. Positions of selected bmCyp-1 residues are also indicated.

3.1. Molecular shape

Discussion of the structural features of the bmCyp-1 is restricted to the refined tetragonal crystal form. The overall architecture of bmCyp-1 is similar to that of the known cyclophilin X-ray and NMR structures [5] with the 8-stranded β-barrel capped by two α-helices. Fig. 2 shows a comparison of hCypA [18] with bmCyp-1. The rmsd between corresponding backbone atoms of 148 residues in hCypA and bmCyp-1 is 0.56 Å. (Divergent loops and N- and C-termini residues were not included.) CypB has higher overall homology and a similar comparison with the X-ray structure using hCypB gives an rmsd fit of 0.52 Å.

The divergent family of cyclophilins contains two highly conserved cysteines (Fig. 1, denoted $). The sulphur atoms of C-43 and C-173 are well defined in the electron density and are separated by 5.43 Å. A small adjustment in the χ_1 torsion angles of both residues would enable a disulphide bond to form. This crystal is apparently composed of the reduced cysteine form.

The amino acid N-terminal fusion derived from the vector multiple cloning site (Ile-Ser-Glu-Phe-Gly-Ser) was not visible

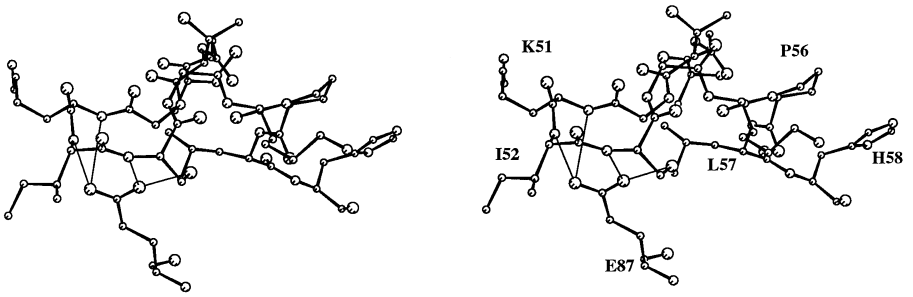
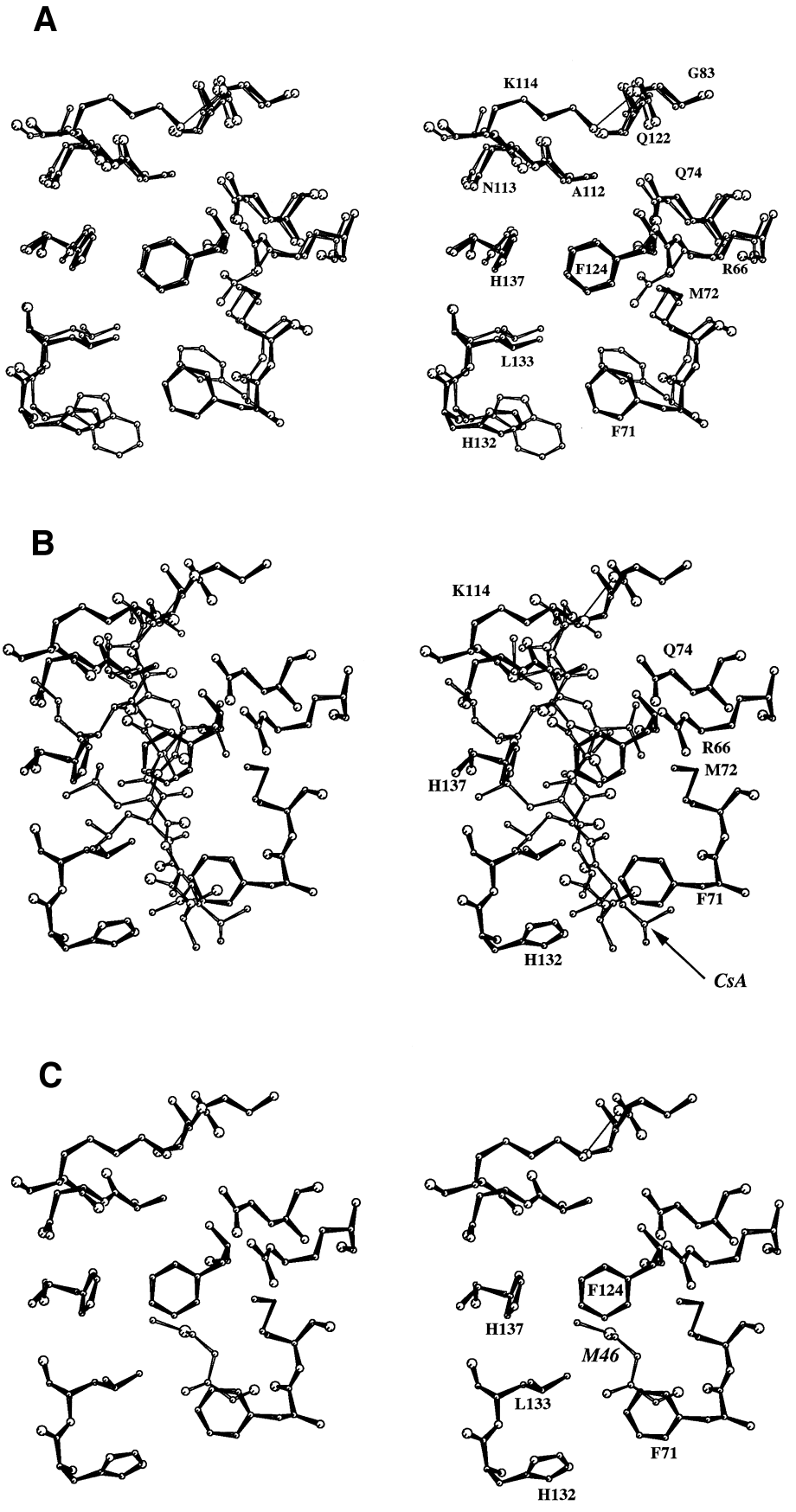


Fig. 3. A stereo view of the octapeptide loop (51-KISGKPLH-58). The four hydrogen bonds from the carboxy group on the side chain of Glu-92 are drawn as thin lines.


A
K114
G83
Q122
N113
A112
Q74
H137
F124
R66
M72
L133
F71
H132
B
K114
Q74
R66
M72
H137
F71
H132
CsA
C
F124
H137
M46
L133
F71
H132

in the final electron density. Mass spectroscopy was used to measure the molecular weight of the protein at 20 095 Da (major peak). This compares with a calculated weight of 20 100 Da for the 183 aa total protein and confirmed that no N-terminal cleavage had occurred.

3.2. Modified loops

The octapeptide loop (51-KISGKPLH-58) provides the most distinctive structural feature differentiating the bmCyp-1 from all other available NMR or X-ray cyclophilin structures [9] (Fig. 1, in italics). The loop is held in place by a tight and very specific network of hydrogen bonds from the side chain of Glu-87 (Fig. 3). Most cyclophilin homologues which have the octapeptide insert (including hCyp-40, hnkCyp, and most plant cyclophilins) have an invariant glutamate in that position while the group of cylophilins without the octapeptide insert have a lysine at that position. This loop is a particularly common feature of the nematode cyclophilins, and is found in 7 of the 11 cyclophilin members from *C. elegans* [2]. The Glu-87 locks the loop into a particular conformation which may be relevant for protein-protein recognition. The rather unusual role of a buried glutamate may even suggest a mechanism forcing a conformational change in the loop on protonation of the glutamate.

Another loop (156-LKTNSKNRP-164) joining α-2 to β-8 has significantly different conformation and sequence in hCypA. This loop also provides a subdivision of cyclophilins in which the sequence (and conformation) of this protruding loop is well conserved in the divergent class of cyclophilins and is significantly different in hCypA as seen in Fig. 2. The difference in conformation in this loop between CypA and CypB has been noted previously [10]. The shape and distribution of charged amino acids on such protruding loops may well provide specific recognition signals for partner proteins in the cell.

3.3. PPIase activity

The PPIase activity of recombinant bmCyp-1 using the substrate succinyl-Ala-Ala-Pro-Phe-p-nitroanilide has a $k_{cat}/K_m = 7.9 \times 10^6$ M^{-1} s^{-1} and is almost as efficient as hCypA which has a $k_{cat}/K_m = 1 \times 10^7$ M^{-1} s^{-1} [22]. A rather high concentration of 860 nM CsA was required to inhibit 50% of the bmCyp-1 PPIase activity. This compares with an IC_{50} value for human CypA of 19 nM [23]. The X-ray structure of the bmCyp-1 cyclophilin domain provides a probable explanation of the poor CsA binding.

The active site of the cyclophilins has been well characterised [9] and the 13 residues involved in binding the large inhibitor CsA are shown in Fig. 1 (denoted *) and highlighted in Fig. 4A, all are conserved in bmCyp-1 with the exception of Lys-114 (usually Ala) and His-132 (usually Trp). It is clear that Lys-114 protruding into the recognition cleft of the PPIase active site of bmCyp-1 will play a significant role in substrate recognition (Fig. 4B). In this crystal structure Lys-114 forms a hydrogen bond with the carbonyl oxygen atom of G72 and the side chain forms a gate-like barrier over the 'Abu-pocket' [5,9], which is one of the key recognition sites for cyclosporin binding. Even in solution it is likely that the steric bulk of the Lys-114 side chain in most low-energy conformations will at least partially block the Abu-pocket. This will prevent tight binding of the bulky CsA ligand and explains the low affinity of the bmCyp-1/CsA complex.

The only other structural differences in the active site of bmCyp-1 compared to that of hCypA are some small conformational changes in the side chains of R66 and F71 (Fig. 4A). It is interesting to note that the four most closely homologous divergent cyclophilins (Fig. 1): bmCyp-1, ceCyp-8, hnkCyp and srCyp all have the Trp to His and Ala to Lys/Arg mutations. In line with these observations, the enzymatically characterised hnkCyp was similarly found to have a low affinity for Csa, with an IC_{50} value of 760 nM [24].

The active site of bmCyp-1 in the tetragonal crystal form is occupied by side chains from a crystallographically neighbouring molecule (Fig. 4C). This packing arrangement is such that the hydrophobic pocket is occupied by the side chain of M46 which forms van der Waals contacts with F71, M72, F124, L133 and H137 which comprise five of the 13 residues involved in CsA binding in hCypA. The sulphur atom of M46 bound in the active site corresponds closely to the position adopted by the valine side chain in Cyp-bound CsA (Fig. 4B, C). This crystal packing arrangement suggests that the Cyp active site may be rather promiscuous and act as a binding site for different hydrophobic amino acid side chains.

3.4. A template for divergent cyclophilins

All four divergent cyclophilin carboxy-terminal domains (bmCyp-1, ceCyp-8, hnkCyp and srCyp) are very basic and are interspersed with serine/arginine- (SR-) rich domains; a common feature also shared with nuclear localisation (NLS) motifs [25]. A bipartite nuclear localisation recognition program [26] showed that all four sequences do indeed contain multiple NLS motifs. Significantly, the srCyp [27] was found to localise to the nuclear speckles, and is thought to be involved in RNA splicing. It is also interesting to note that the terminal region of the *Brugia* and *C. elegans* cyclophilins both contain a large continuous run of serine residues (data not shown).

We suggest that the *C. elegans* Cyp-8 isoform is a direct homologue of bmCyp-1 and therefore information resolved from this genetically amenable nematode can be directly related to the nematode parasite *B. malayi*. For instance, we now have evidence via *Cyp-8* promoter, reporter gene analysis that this divergent homologue is expressed in the gut cell nuclei of *C. elegans* and has a consistent expression pattern

←

Fig. 4. A: Stereo view of an overlay of the active site of bmCyp-1 (filled bonds) with hCypA (unfilled bonds) showing the side chains of the 13 residues known to be involved in substrate and inhibitor binding of hCypA. The hydrogen bond from the amino nitrogen atom of K114 to the carbonyl O of G83 is shown as a thin line. B: Stereo view of the active site of bmCyp-1 (filled bonds) with the cyclic peptide cyclosporin A (CsA). CsA coordinates are from the hCypA/CsA complex [18] and positioned from the least squares fit used in A. The side chain of K114 in the bmCyp-1 structure acts as a gate to block access to the 'Abu-pocket' which is a deep accessible cleft in the hCypA structure. C: Stereo view of the active site of bmCyp-1 (filled bonds) showing the position M46 (unfilled bonds) from a crystallographically related neighbouring molecule. M46 forms van der Waals contacts with F71, M72, F124, L133 and H137 and suggests that the hydrophobic active site of cyclophilins may accommodate a range of large hydrophobic groups.

throughout the life cycle, from embryo to adult (Page, unpublished results).

The structural differences in the active site of this divergent class of cyclophilins, particularly prevalent in nematodes, provides a useful starting point for the development of specific ligands which may have potential as antiparasitic drugs.

Acknowledgements: The authors would like to thank Dr. Mario Philipp, Tulane Primate Center, LA, USA for the kind gift of the *B. malayi* adult male cDNA library. A.P.P. is funded by the MRC (Great Britain) through a Career Development Award. G.K. is sponsored by a studentship from Novartis AG.

References

[1] Galat, A. and Metcalfe, S.M. (1995) Prog. Biophys. Mol. Biol. 63, 67–118.
[2] Page, A.P., Macniven, K. and Hengartner, M.O. (1996) Biochem. J. 317, 179–185.
[3] Hsu, V.L., Heald, S.L., Harding, M.W., Handschumacher, R.E. and Armitage, I.M. (1990) Biochem. Pharmacol. 40, 131–140.
[4] Davis, J.M., Boswell, B.A. and Bachinger, H.P. (1989) J. Biol. Chem. 264, 8956–8962.
[5] Braun, W., Kallen, J., Mikol, V., Walkinshaw, M.D. and Wuthrich, K. (1995) FASEB J. 9, 63–72.
[6] Schreiber, S.L. (1993) FASEB J. 7, A1054.
[7] Page, A.P., Kumar, S. and Carlow, C.K.S. (1995) Parasitol. Today 11, 385–388.
[8] Chappell, L.H. and Wastling, J.M. (1992) Parasitology 105, S25–S40.
[9] Taylor, P., Husi, H., Kontopidis, G. and Walkinshaw, M.D. (1997) Prog. Biophys. Mol. Biol. 67, 155–181.
[10] Mikol, V., Kallen, J. and Walkinshaw, M.D. (1994) Proc. Natl. Acad. Sci. USA 91, 5183–5186.
[11] Nicolli, A., Basso, E., Petronilli, V. and Bernardi, P. (1996) Biophys. J. 70, TUAM6.
[12] Warth, R., Briand, P.A. and Picard, D. (1997) Biol. Chem. 378, 381–391.
[13] Ratajczak, T. and Carrello, A. (1996) J. Biol. Chem. 271, 2961–2965.
[14] Page, A.P., Landry, D., Wilson, G.G. and Carlow, C.K.S. (1995) Biochemistry 34, 11545–11550.
[15] Dennis, D.T., Dreyer, G., Ismail, M.M., Kumaraswami, V., Mak, J.W., Mataika, J.U., Ottesen, E.A., Piessens, W.F., Rajagopalan, P.K., Southgate, B.A. and Huijin, Z. (1992) WHO Tech. Rep. Ser. 1, 71.
[16] Otwinowski, Z. and Minor, W. (1993) in: L. Sawyer, N. Isaacs and S. Baily (Eds.), Data Collecting and Processing Proceedings of the CCP4 Study Weekend, 56–62.
[17] Navaza, J. (1994) Acta Crystallogr. A50, 157–163.
[18] Mikol, V., Kallen, J., Pflugl, G. and Walkinshaw, M.D. (1993) J. Mol. Biol. 234, 1119–1130.
[19] Sheldrick, G.M. (1997) SHELX-97, Abstract.
[20] Brunger, A.T. (1993) X-PLOR version 3.1: a system for X-ray crystallography and NMR, Abstract.
[21] Jiang, J.S. and Brunger, A.T. (1994) J. Mol. Biol. 243, 100–115.
[22] Liu, J. and Walsh, C.T. (1990) Proc. Natl. Acad. Sci. USA 87, 4028–4032.
[23] Liu, J., Albers, M.W., Chen, C.M., Schreiber, S.L. and Walsh, C.T. (1990) Proc. Natl. Acad. Sci. USA 87, 2304–2308.
[24] Rinfret, A., Collins, C., Menard, R. and Anderson, S.K. (1994) Biochemistry 33, 1668–1673.
[25] Valcarcel, J. and Green, M.R. (1996) Trends Biochem. Sci. 21, 296–301.
[26] Robbins, J., Dilworth, S.M., Laskey, R.A. and Dingwall, C. (1991) Cell 64, 615–623.
[27] Bourquin, J.P., Stagljar, I., Meier, P., Moosman, P., Silke, J., Baechi, T., Georgiev, O. and Schaffner, W. (1997) Nucleic Acids Res. 25, 2055–2061.