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Structure and dynamics of the anticodon-arm binding domain of *Bacillus stearothermophilus* tyrosyl-tRNA synthetase

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Running head: TyrRS C-Terminal Domain - Structure and Dynamics

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Summary

Background: The accuracy of aminoacyl-tRNA synthetases in tRNA charging is crucial for protein biosynthesis. Most synthetases have a dedicated structural domain which specifically interacts with the anticodon-arm of the cognate tRNAs and is essential for correct charging. In the crystal structure of *Bacillus stearothermophilus* tyrosyl-tRNA synthetase (TyrRS), the anticodon-arm binding domain (C-terminal domain) appears disordered.

Results: The solution structure of a recombinant protein, TyrRS(4), corresponding to the C-terminal domain of *B. stearothermophilus* TyrRS, has been solved and its dynamics studied by NMR. The structure consists of a 5-stranded β -sheet, packed against two α -helices on one side and one α -helix on the other side. Order parameters and previous data suggest that the disorder observed in the crystals is due to a flexible linker between the N- and C- terminal domains. A large part of the domain is structurally similar to other functionally unrelated RNA binding proteins. The basic residues known to be essential for tRNA binding and charging are exposed to the solvent on the same face of the molecule.

Conclusions: The structure of TyrRS(4), together with previous mutagenesis data, allows one to delineate the region of interaction with tRNA^{Tyr}. It is the first structure described for an anticodon-arm-binding domain of a tyrosyl-tRNA synthetase. It completes the structure of the *B. stearothermophilus* enzyme and will help to understand its mechanism of action.

Introduction

Aminoacyl-tRNA synthetases are the enzymes that translate the genetic code *in vivo*. Each synthetase specifically links an amino acid to its anticodons through the charging of the cognate tRNAs. The amino acid is first activated with ATP to form an aminoacyl-adenylate and then transferred from this intermediate to the acceptor end of the tRNA [1]. The synthetases are modular proteins. In addition to their catalytic domain, whose fold is conserved and belongs to one of two classes, most synthetases possess one or two idiosyncratic domains [2]. These latter domains

specifically recognise the anticodon arm of the cognate tRNA and are of utmost importance for the accuracy of charging.

Tyrosyl-tRNA synthetase (TyrRS) is a homodimeric protein that catalyses the formation of tyrosyl-tRNA^{Tyr}. The crystal structure of TyrRS from *B. stearothermophilus* has been solved at 2.3 Å resolution [3]. Each monomer comprises three domains: (i) the catalytic / domain (residues 1-247), which contains the binding sites for tyrosine, the tyrosyl-adenylate intermediate and the acceptor stem of tRNA^{Tyr}, as well as the dimerisation interface; (ii) the -helical domain (248-319) with at one end a catalytic loop, and at the other end, residue F₃₂₃, which interacts with tRNA^{Tyr} and may be involved in the specific recognition of the anticodon [4]; (iii) the C-terminal domain (C-TyrRS, residues 320-419) which shows a very low electron density that hampers the tracing of its polypeptide chain. Experiments with truncated homo- and heterodimers lacking the C-terminal domain have shown that C-TyrRS is necessary for tRNA^{Tyr} binding and charging, and that one tRNA^{Tyr} molecule binds to the C-terminal domain of one monomer and to the N-terminal / domain of the other monomer [5, 6]. Site-directed mutagenesis experiments have identified six basic residues (R₃₆₈, R₃₇₁, R₄₀₇, R₄₀₈, K₄₁₀, K₄₁₁) that are necessary for tRNA^{Tyr} charging [7]. The recombinant protein TyrRS(4) contains residues 320-419, and a Leu-Glu-His₆ C-terminal extension. It therefore corresponds to an isolated C-TyrRS domain. TyrRS(4) behaves as a folded globular monomeric protein in solution, and circular dichroism experiments have indicated that its structure is effectively identical to that of C-TyrRS in the context of the full-length synthetase [8-10]. The secondary structure of TyrRS(4) is novel among the anticodon-arm binding domains of synthetases [9]. Here, we report the three-dimensional solution structure of TyrRS(4) and its backbone dynamics determined by NMR.

Results and Discussion

Structure Description

The structure of TyrRS(4) is composed of a 5-stranded β -sheet, flanked on one side by two α -helices that run roughly antiparallel to one another, and on the other side by a third α -helix (Figure 1, Table 1).

The 9 N-terminal residues of TyrRS(4) are disordered, specially the first 5 residues which showed no nOes with the rest of the protein or whose amide NMR signals were not observed. The first α -helix (α_1 :332-339) is connected by a hairpin loop to a β -strand (β_1 : 344-347). A less well defined loop, centred at residue 349, leads to two antiparallel α -helices (α_2 : 354-361 and α_3 : 367-375) that are followed by four strands of the β -sheet (β_2 : 379-381, β_3 : 384-385, β_4 : 404-408, and β_5 : 413-418). Strands β_2 and β_3 are linked by a short 2-residue turn, while strands β_3 and β_4 are connected by a long, meander-shaped loop (386-403) with a short helical segment (α_4 : 395-397) in the middle. Except for α_1 , which runs parallel to β_5 , the arrangement of the sheet strands is antiparallel.

Helix α_1 and the β -sheet are packed against each other through hydrophobic interactions that involve A₃₃₂, I₃₃₅, and F₃₃₉ in helix α_1 and V₄₀₅, Y₄₁₃, Y₄₁₄ and L₄₁₅ in strands β_4 and β_5 . Residues L₃₃₀, V₃₄₂, N₃₈₂ and F₄₀₃ also make important contributions to the packing interactions on this side of the sheet. The main hydrophobic core of the protein is essentially formed by the packing of helix α_2 , helix α_3 and the other side of the β -sheet (strands β_2 , β_4 , β_5). The hydrophobic residues belonging to secondary structure elements and contributing to this core include L₃₅₄, L₃₅₇, L₃₅₈ and V₃₅₅ from helix α_2 , A₃₇₀ and I₃₇₄ from helix α_3 , I₃₇₉ and V₃₈₁ from strand β_2 , and I₄₀₆ and I₄₁₆ from strands β_4 and β_5 , respectively. Residues I₃₆₃, I₃₉₂ and A₃₉₅, located in loop regions, also participate in this core. Finally, helices α_2 and α_3 are held together mainly by hydrophobic interactions between L₃₅₄, V₃₅₅, L₃₅₈, V₃₅₉, A₃₇₀ and I₃₇₄.

Correlating the Backbone Dynamics and the Structure

The N and C termini of TyrRS(4) show low values of the order parameter S^2 , which indicate high amplitude motions on the ps-ns time scale (Figure 2). In contrast, the rest of the protein displays high S^2 values (except for residues G₃₄₉ and G₃₅₀ in loop α_1 - α_2), typical of globular proteins. All residues within the helices and β -sheet have S^2 values ca. 0.80, while some residues in loops show slightly lower values. The S^2

and backbone RMSD values of the structural ensemble are inversely correlated for the N and C termini, as well as for loop 1-2. These correlations indicate that internal motions on the ps-ns time scale are responsible for the structural variability observed in these regions. Loop 4-5 has high RMSD values that could also be due to the dynamics of the protein. Indeed, no amide NMR signals were detected for K₄₁₀ and K₄₁₁, probably because of exchange broadening. The remaining regions showing RMSD values significantly higher than the mean are centred at residues E₃₄₁, G₃₈₃, G₃₉₀, E₃₉₆ and E₄₀₀. These residues have high S² values. Such values indicate the absence of fast motions of high amplitude and suggest that the higher RMSD values are due to the reduced number of experimental restraints in these solvent-exposed loop regions. Finally, high R_{ex} rates, indicative of slow local conformational exchange on the μs-ms time scale, are observed in helices 1 and 3, as well as in loop regions.

Possible Cause of the Crystallographic Disorder of C-TyrRS

The disorder observed for C-TyrRS in the crystals of the full-length protein [3] could be of either static origin (same structure of C-TyrRS at different positions within the lattice) or dynamic origin (high mobility within the domain). ¹⁵N relaxation data show that the N-terminal residues of TyrRS(4) are disordered and highly mobile while the rest of the molecule displays typical dynamics of a well-ordered and structured globular protein. These observations suggest that the disorder observed in the crystals is of static origin and that it is due to the flexibility of the peptide linking the α -helical and C-terminal domains. In the context of the full-length protein, interactions of the linker and/or C-TyrRS with the rest of the protein could restrict its mobility. Available data, however, do not support this latter possibility. Indeed, (i) the N- and C-terminal fragments (residues 1-317 and 320-419, respectively) can fold independently into entities that are stable under conditions similar to those used for crystallisation; (ii) the structures of the α / β and α -helical domains are identical in the crystals of either the full-length protein or the N-terminal fragment [3, 11]; (iii) the structure of the C-terminal domain is effectively the same whether this domain is isolated in solution (TyrRS(4)) or present in the context of the full-length TyrRS, as revealed by circular dichroism in the far and near UV regions [10]; (iv) double hybrid

experiments failed to show any interaction between the C- and N-terminal fragments [12]; (v) several TyrRS insertion mutations, containing up to five residues in the linker region (position 325), had no significant effect on its specific activity [4]. As these insertions were rich in glycines, this result indicates that the linker can be flexible (and of variable length) without compromising the aminoacylation activity of TyrRS. Taken together, these arguments strongly suggest that the linker region is flexible in TyrRS, and that the C-terminal domain does not interact strongly with the remainder of the protein. TyrRS would thus be the only aminoacyl synthetase that has not evolved strong interactions between its anticodon-arm-binding domain and its catalytic domain.

Structural Similarity between TyrRS($\Delta 4$) and other RNA Binding Proteins

Sequence alignments have predicted that the C-terminal domains of eubacterial tyrosyl-tRNA synthetases contain the so-called "S4 motif", which is also present in the proteins of several families with diverse functions [13-15]. The role of this motif would be to display positively charged residues for interaction with the phosphates of an RNA ligand [15]. Accordingly, when the coordinates of the TyrRS($\Delta 4$) structure were submitted to the server DALI [16], three structures containing the S4 motif showed significant homologies with TyrRS($\Delta 4$) (statistical Z score > 2.0). These were: the *Escherichia coli* ribosome-binding heat-shock protein Hsp15 (Z = 4.5), the ETS domain of *B. stearothermophilus* ribosomal protein S4 (Z = 4.7) and, to a lesser extent, the N-terminal domain N1 of the *E. coli* threonyl-tRNA synthetase (ThrRS, Z = 2.4), whose function is unknown [13, 15, 17]. Despite low sequence identity (< 20 % relative to C-TyrRS), the above domains display a common fold, consisting of a three- or four-stranded antiparallel β -sheet packed against two α -helices (Figure 3). A comparison of the structures of Hsp15, S4 and ThrRS has previously revealed a structurally similar region between α_2 and α_4 (TyrRS($\Delta 4$) numbering), the L motif [15]. Most of the residues conserved across the families are in the α_2 - α_3 and α_2 - α_3 regions and some of these are exposed to the solvent. The buried residues involved in packing helices α_2 and α_3 together are particularly similar in the four proteins. The main structural differences between TyrRS($\Delta 4$) and the other proteins are located in

the long α_3 - α_4 loop. The length of this loop in TyrRS(4) and its low sequence similarity may explain these differences.

Comparison with other Eubacterial TyrRS C-terminal Domains

The sequences of 27 C-TyrRS domains from eubacteria were retrieved and aligned as described in Ref. [4]. The main features of this alignment are summarised on the sequence of *B. stearothermophilus* C-TyrRS in Figure 3e. The conserved residues mainly belong to the region between α_2 and α_3 , which is included in the S4 motif, and to the region between α_4 and α_5 , rich in basic residues. All the hydrophobic residues that contribute to the main core of C-TyrRS (see above) are conserved, except I₃₉₂ and A₃₉₅ in the long α_3 - α_4 loop. In particular, all the buried residues involved in interactions between helices α_2 and α_3 are conserved (50% identity), and these are also very similar in the other RNA binding protein families. Therefore, the interactions which contribute to the packing of helices α_2 and α_3 together and against one side of the sheet appear important to preserve the S4 motif, while the α_3 - α_4 loop seems less important. The conservation of hydrophobic residues also suggests that the S4 motif is preserved among the eubacterial TyrRSs. In contrast, several residues involved in packing the protein on the other side of the sheet are not conserved. Finally, only L₃₂₂, L₃₃₀, and I₃₃₅ are conserved between residues 321 and 340, together with the functionally essential residue F₃₂₃ [4].

tRNA Binding

In vitro tRNA charging and *in vivo* complementation experiments have shown that six basic residues of the C-terminal domain are important for the interaction of TyrRS with tRNA^{Tyr} [7]. These residues are highly exposed to the solvent, except for the non-conserved residue R₄₀₈, which is less exposed. The six residues lie on the same face of the molecule and constitute a highly positive surface that can bind the negatively charged tRNA (Figures 4a and 4b). They are located in two separate regions. The first one involves R₃₆₈ and R₃₇₁, the latter residue being conserved across the four families of RNA binding proteins. Interestingly, S4 and Hsp15 show conserved residues within a positively charged patch in an equivalent spatial region. In the case of S4, this region contacts the 16S ribosomal RNA [18]. Residues R₄₀₇,

R₄₀₈, K₄₁₀ and K₄₁₁, located in strands ₄ and ₅, constitute the second positive region, which is conserved among the TyrRSs but has no equivalent in S4 or Hsp15. This suggests that, in TyrRS, the general S4 motif is complemented by an idiosyncratic motif to specifically recognise tRNA^{Tyr}.

Five other basic residues have been mutated in the C-terminal domain of TyrRS and found to be irrelevant for *in vivo* tRNA^{Tyr} charging in complementation experiments [7]. Three of these, R₄₀₂, R₄₁₇ and R₃₉₈, lie on the opposite face of the molecule, indicating that only one face of the molecule is implicated in the interaction with tRNA^{Tyr} (Figure 4c). These residues form two positive patches in an otherwise rather negative surface. The other residues, K₃₆₇ and R₃₈₅, are on two edges of the binding face.

Several residues for which no experimental data is available, are exposed on the face of C-TyrRS that interacts with tRNA^{Tyr} (Figure 4d). Some are conserved among eubacteria and could participate in tRNA binding through ionic interactions, hydrogen bonds or aromatic-ring stacking with bases: S₃₆₆, Q₃₇₅, N₃₇₆, G₃₇₇, G₄₀₉, K₄₁₂ and Y₄₁₃. Interestingly, mutation S₃₅₆->A in *Acidobacillus ferrooxidans* TyrRS (S₃₅₆ is equivalent to S₃₆₆ in *B. stearothermophilus*), increases significantly its K_M for tRNA^{Tyr} [19]. Mutagenesis experiments of these residues and/or the structure of the complex of TyrRS with its cognate tRNA should help in establishing the relevance of these residues for the interaction.

Biological Implications

The C-terminal domain of TyrRS, which interacts with the anticodon-arm of tRNA^{Tyr}, is essential for binding and charging tRNA^{Tyr}. The structure of TyrRS(4) presented here is the first described for the C-terminal domain of a tyrosyl-tRNA synthetase and shows a novel fold among the anticodon-arm binding domains of aminoacyl-tRNA synthetases. The structure contains the S4 motif, which is also present in other families of RNA binding proteins. The conservation profile of residues involved in maintaining the architecture of TyrRS(4) indicates that this structure represents a prototype for the C-terminal domain of the eubacterial TyrRSs.

TyrRS(4) displays a face rich in positive residues which interacts with the

negatively charged tRNA. Six of these residues have, indeed, previously been shown by mutagenesis to be important for tRNA^{Tyr} binding. The other evolutionary conserved residues on this face may interact with phosphate, ribose or base moieties of tRNA^{Tyr}. The structure thus allows one to rationalise previous mutagenesis data and to pinpoint further mutagenesis sites. The conservation of solvent exposed residues on the binding face of TyrRS(4) suggests that the C-terminal domain of the other eubacterial TyrRSs bind their cognate tRNA^{Tyr} by similar mechanisms.

The structure of TyrRS(4) completes that of the free enzyme from *B. stearothermophilus* for which only the structure of the N-terminal region could be solved by X-ray crystallography [3]. Whenever the structure of a complex between TyrRS and tRNA^{Tyr} is available, it will be possible to compare the structures of the free and bound C-terminal domain and thereby establish whether conformational changes are involved in the interaction.

Experimental Procedures

Sample Preparation

¹⁵N and ¹⁵N-¹³C labelled recombinant TyrRS(4) was expressed in *E. coli* and purified as described [9]. Samples were prepared in 20 mM potassium phosphate buffer pH 6.8 with a protein concentration ranging from 0.8 to 1.2 mM.

NMR

NMR experiments were run at 35 °C on a Varian Inova spectrometer resonating at a 499.83 MHz ¹H frequency. Vnmr (Varian Inc.) and XEASY [20] were used for data processing and analysis. ¹H, ¹⁵N and ¹³C sequential assignments were achieved using a combination of triple resonance CBCA(CO)NH and HNCACB experiments [21] and of three-dimensional ¹⁵N-edited NOESY-HSQC and TOCSY-HSQC spectra [22]. ¹H and ¹³C side-chain assignments were performed using 3D H(CC-TOCSY)NNH, C(CC-TOCSY)NNH [23, 24], 3D ¹³C-edited HCCH-TOCSY [25], ¹⁵N-edited TOCSY-HSQC [22], 2D ¹H-¹H DQF-COSY [26, 27] and 2D experiments to correlate aromatic protons with C carbons [28].

Distance constraints were derived from a 3D ^{15}N -edited NOESY-HSQC spectrum recorded in H_2O with a 150 ms mixing time, as well as from a 2D ^1H - ^1H NOESY spectrum acquired in D_2O with a mixing time of 100 ms. The latter NOESY was acquired on a 800 MHz Bruker DRX-800 spectrometer. NOe intensities were evaluated from peak heights and calibrated using the CALIBA routine of DYANA [29]. J_{HNH} coupling constraints were obtained from a HMQC-J [30] spectrum as described [9] and converted to constraints for dihedral angles as follows: (-90° , -40°) for $J_{\text{HNH}} < 5.5$ Hz and (-160° , -80°) for $J_{\text{HNH}} > 8$ Hz.

^{15}N Relaxation Measurements and Analysis

Longitudinal (R_1) and transverse (R_2) ^{15}N amide relaxation rates, as well as ^{15}N - ^1H nOe data were obtained with pulse schemes described by Kay and coworkers [31]. Nine relaxation-time data points were used to determine R_1 (60 to 1000 ms) and R_2 (10 to 190 ms). R_1 and R_2 data were fitted to monoexponential decays without offset. Error on data points was estimated as 4 (R_1 and R_2) or 3 (nOe) noise RMSD's. Relaxation data were analysed using the extended [32] Lipari and Szabo formalism [33] with MODELFREE version 4.1 [34, 35]. The statistical approach to model selection [35] was followed. Isotropic tumbling was assumed as the ratio of the parallel and perpendicular axes of the diffusion tensor [36] was very close to unity (1.080 ± 0.008).

NOe Assignments and Structure Calculations

Starting from 639 manually assigned peaks (mostly intraresidual, sequential and secondary-structure related nOes), a total number of 2017 nOe peaks from the ^{15}N -edited NOESY-HSQC (H_2O) and from the 2D NOESY (D_2O) spectra were assigned using 48 cycles of simulated annealing within NOAH [37]. NOe assignments were carefully inspected and completed manually. This procedure resulted in 1352 meaningful upper distance constraints. Experimental constraints included also 71 dihedral angles and 33 backbone-backbone hydrogen bonds. A hydrogen-bond constraint was added only when 67 % of the preliminary structures showed a hydrogen bond and this was in agreement with saturation transfer [9] and hydrogen exchange in D_2O experiments.

From the 150 structures calculated using the torsion angle dynamics protocol in DYANA [29], the 50 structures with the lowest target function value were subjected to restrained energy minimisation in water using OPAL [38] with the AMBER94 force field. The 20 structures with the lowest total energy values were selected as representative of the TyrRS(4) structure (Table 1). Structures were displayed and analysed with MOLMOL [39]; their quality was evaluated using PROCHECK [40].

Supplementary material

A table showing relaxation (R_1 , R_2 , nOe) and "model-free" (order parameters, R_{ex} , internal correlation time) parameters.

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Accession Numbers

The atomic coordinates of the structure of TyrRS(4) have been deposited with the RCBS protein data bank (PDB code: 1JH3); chemical shift data have been deposited with the BioMagResBank (accession code: 5070).

Figure 1. Structure of TyrRS(4)

(a) Ribbon drawing of one conformer chosen to represent the structural ensemble. N and C, N- and C-terminus, respectively. The disordered N-terminal residues are not shown. (b) Secondary structure topology. Helices are shown in red and α -strands in cyan as in (a). Numbers indicate their starting and ending residues. The size of a rectangle does not accurately represent the relative length of the corresponding secondary structure element. (c) Stereo view of the backbone superposition of the 20-conformer structural ensemble.

Figure 2. TyrRS(4) Backbone Dynamics, RMSD of the Calculated Structural Ensemble and the NOEs Used for Obtaining the Structures

(a) Order parameter (S^2). S^2 reflects the amplitude of fast internal motions of the NH vector in the ps-ns time scale and varies between 0 (high amplitude motions) and 1 (rigid body). (b) Backbone (C', N and C) RMSD from the mean structure after best superposition of each structure to the mean structure between residues 330-418. (c) Number of meaningful nOes between residues i and j used as constraints in structure calculations; white: intraresidue ($j = i$); light-grey: sequential ($j = i + 1$); dark-grey: medium range ($i + 2 \leq j \leq i + 4$); black: long range ($j \geq i + 5$). (d) Rate of conformational exchange (R_{ex} , in s^{-1}) that indicates slow conformational exchange on the μs -ms time scale. S^2 and R_{ex} values were obtained using an isotropic rotational correlation time of 6.85 ns. Error bars are displayed for these parameters.

Figure 3. Comparison of TyrRS(4) with other RNA Binding Proteins

Ribbon diagram of the structure of (a) TyrRS(4), (b) Hsp15 (Z score = 4.5, C RMSD = 2.2 Å over 62 residues), (c) S4 (Z score = 4.7, C RMSD = 2.1 Å over 60 residues) and (d) the N1 domain of ThrRS (Z score = 2.4, C RMSD = 2.5 Å over 59 residues). Only the regions with structural homology are shown. The region 144-171 of S4 that is absent in the other proteins is shown in green. (e) Sequence alignment based on structure superposition obtained from the server DALI [16]. The secondary structure of TyrRS(4) is represented on top of the alignment and the sequences of corresponding secondary structure elements, as determined by MOLMOL [39], are shaded in grey. Residues 146-170 of S4 are not represented. The short α -strands

11-13 and 55-56 of ThrRS belong to another β -sheet and should not be taken as part of the S4 module. These strands are not represented in (d). Residues in lower case were not considered to obtain the alignment. Structurally-similar conserved or identical (≥ 50 %) residues within each family are coloured in red. The highly conserved (≥ 85 % identical) residues of TyrRS are underlined. Residues that are similar or identical in at least 3 of the proteins are boxed. Residue-conservation information for Hsp15, S4 and ThrRS is taken from Ref. [15].

Figure 4. Surface Representations of the Structure of TyrRS(4) (Residues 330-418) A ribbon diagram is displayed at the centre of the figure to show the orientation of the molecule used in the surface representations. (a) The six basic residues identified by mutagenesis as essential for interaction with tRNA^{Tyr} are shown in blue, while mutated residues that are not relevant to tRNA interaction as assessed by an in vivo genetic complementation assay [7] are represented in orange. Different blues are used for clarity. (b) and (c), Surface electrostatic potential of TyrRS(4) in the same orientation as in (a) and after a 180 ° y rotation, respectively. Positive and negative potentials are represented in blue and red, respectively. Electrostatic potentials were calculated with MOLMOL [39]. (d) Analysis of the putative binding surface. The basic residues known to be important (blue) or irrelevant for tRNA binding (orange) shown in a, are displayed without label. The remaining residues on the tRNA binding face are labelled and coloured: red, negatively charged residues that most probably do not interact with tRNA; cyan, positively charged residues that could in principle interact with tRNA phosphates; purple and violet, polar residues (purple) and glycines (violet) with an exposed amide group that could form hydrogen bonds with tRNA bases or ribose; yellow, exposed aromatic residues that could stack with tRNA bases; magenta, A₃₇₈.

Table 1. Statistics of the NMR Structural Ensemble of TyrRS(4)

Parameter	Value
DYANA:	
Number of nOe upper distance limits ^a	1352
Number of dihedral angle constraints	71
Number of hydrogen bonds	33
Residual target function (\AA^2) (mean value for 50 conformers)	5.72 ± 0.66
OPAL: average for the best 20 conformers	
Residual distance constraint violations	
Number 0.1 \AA	3.90 ± 2.12
Maximum (\AA)	0.10 ± 0.01
Residual dihedral angle constraint violations	
Number 2.0 $^\circ$	0.55 ± 0.59
Maximum ($^\circ$)	2.01 ± 0.24
AMBER energies (kcal/mol)	
Total	$-3,974 \pm 54$
Van der Waals	-247 ± 10
Electrostatic	$-4,645 \pm 66$
Mean pairwise RMSD (\AA) ^b	
Backbone atoms N, C , C '(330-418)	0.57 ± 0.09
Heavy atoms (330-418)	1.38 ± 0.19
Ensemble Ramachandran plot:	
Residues in most favoured regions	68.6 %
additional allowed	29.3 %
generously allowed	1.8 %
disallowed regions	0.4 %

^a Unambiguous meaningful nOes used for structure calculations (389 long range, 297 medium range, 372 sequential and 294 intraresidue nOes).

^b Mean of the pairwise RMSD between residues 330-418, thus excluding the flexible N and C-termini.