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Structural analysis of interdomain mobility in ribosomal L1 proteins

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Ribosomal protein L1 consists of two domains connected by two oppositely directed fragments of the polypeptide chain in a hinge-resembling fashion. The domain arrangement determines the overall shape of the protein, corresponding to an open or a closed conformation. Ribosomal L1 proteins from archaea demonstrate the open conformation in both isolated and RNA-bound forms. RNA-free ribosomal L1 proteins from bacteria display the closed conformation, whereas in complex with RNA these proteins exist in an open conformation similar to their archaeal counterparts. Analysis of all available L1 amino-acid sequences shows that in comparison to the archaeal proteins, the bacterial proteins possess an extra residue in one of the two interdomain fragments which could be responsible for their closed conformation. To verify this suggestion, a *Thermus thermophilus* L1 mutant lacking one residue in the fragment corresponding to the hinge was obtained and its crystal structure was solved. It was found that this mutation transformed the closed conformation of the bacterial L1 protein into an open conformation similar to that of the archaeal L1 proteins.

1. Introduction

Ribosomal protein L1 is an important primary 23S rRNA-binding protein as well as a translational repressor binding its own mRNA. To date, several crystal structures of L1 proteins from bacteria and archaea have been determined in RNA-free (Nikonov *et al.*, 1996; Unge *et al.*, 1997; Nevskaya *et al.*, 2000, 2002; Nikonova *et al.*, 2011) and RNA-bound (Nikulin *et al.*, 2003; Nevskaya *et al.*, 2005, 2006; Tishchenko *et al.*, 2006; Gao *et al.*, 2009) forms.

L1 is a two-domain protein in which the N- and C-termini are located in domain I. This domain demonstrates an α/β structure, while domain II has a version of the Rossmann-fold topology. Two oppositely directed polypeptide fragments of different lengths connect the two domains in a hinge-resembling fashion.

In all L1 structures both domains retain their fold, but there are differences in the relative domain orientation. Archaeal L1 proteins demonstrate the so-called open conformation both in the isolated form (Nevskaya *et al.*, 2000, 2002) and in complexes with 23S rRNA (Nikulin *et al.*, 2003) and mRNA (Nevskaya *et al.*, 2005; Tishchenko *et al.*, 2006). In this conformation the two domains of L1 are quite separated from each other and the RNA-binding region of the protein is accessible to RNA. A similar conformation was found for L1 from the bacterium *Thermus thermophilus* (TthL1) in complex with mRNA (Nevskaya *et al.*, 2006; Tishchenko *et al.*, 2006). In contrast, in the RNA-free form the two domains of TthL1 approach each other and the protein attains the so-called

closed conformation in which domain II shields the RNA-binding region of domain I (Nikonov *et al.*, 1996). The recently determined structure of L1 from another bacterium *Aquifex aeolicus* (AaeL1) also displayed a closed conformation of the molecule in the RNA-free form (Nikonova *et al.*, 2011). The closed conformation is characterized by an unusually small interdomain contact surface and both few and weak interdomain interactions (Nikonov *et al.*, 1996). This makes interdomain movement in the bacterial L1 proteins a likely event and enables the protein to bind RNA.

To reveal the reason for the higher interdomain mobility of bacterial L1 proteins compared with archaeal L1 proteins, we analyzed all available L1 amino-acid sequences and found an extra residue in the short fragment of the hinge in the bacterial L1 proteins. We proposed that shortening of this fragment would force the mutant to assume the open conformation in the RNA-free form similar to that of the archaeal L1 proteins. To test such a suggestion, the mutant TthL1(-A) lacking Ala158 was produced and its crystal structure was solved. As expected, the obtained protein demonstrated an overall molecular shape that was closely related to the open conformation of L1 proteins from archaea.

Table 1
Data-collection and refinement statistics for TthL1(-A).

Data were collected at 110 K. Values in parentheses are for the highest resolution shell.

Data collection	
Wavelength (Å)	0.91841
Space group	$C222_1$
Unit-cell parameters (Å)	$a = 63.6$, $b = 104.5$, $c = 64.3$
Resolution (Å)	40.0–1.95 (2.0–1.95)
Measured reflections	61736 (2979)
Unique reflections	15656 (947)
Completeness (%)	97.9 (85.3)
Multiplicity	3.9 (3.15)
$\langle I/\sigma(I)\rangle$	11.94 (1.78)
$R_{\rm merge}$ (%)	8.7 (68.4)
Mosaicity (°)	∼0.2
Refinement	
Resolution (Å)	32.1–1.95 (2.07–1.95)
No. of reflections	15652 (2402)
R factor (%)	18.4 (25.5)
Free R factor (%)	22.6 (32.1)
Overall B factor (Å^2)	27.6
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	1.08
No. of solvent molecules	90
Ramachandran plot	
Most favourable (%)	92.6
Additionally allowed (%)	7.4
Generously allowed (%)	0.0

2. Materials and methods

2.1. Plasmid construction

We used QuikChange site-directed mutagenesis to clone a TthL1 gene lacking Ala158, TthL1(-A). pTthL1.4 (Tishchenko *et al.*, 2007), carrying the TthL1 gene, was used as a PCR template. The mutagenesis primers for TthL1(-A) were sense, 5′-CATCCGGGAGATCAAGGGGCGGATTG-3′, and antisense, 5′-GAACTCAATCCGCCCCTTGATCTCC-3′. These primers were extended during temperature cycling by KOD Hot Start DNA polymerase, generating the mutated plasmid. After temperature cycling, the products were treated with endonuclease *Dpn*I. The obtained vector containing the desired mutation was then transformed into XL1-Blue competent cells. The nucleotide sequence of the gene was verified by sequencing.

2.2. Protein overproduction, purification and crystallization

TthL1(-A) was overproduced in *Escherichia coli* BL21 (DE3) cells. Purification of the mutant protein was performed as described for wild-type TthL1 (Nikonov *et al.*, 1996) with an additional step of purification of the protein on heparin Sepharose. Crystallization conditions were found by random screening.

Crystallization experiments were performed at 277 K using the hanging-drop vapour-diffusion method on siliconized glass cover slides in Linbro plates. Drops were made by mixing protein solution at 11 mg ml^{-1} in 30 m*M* Tris–HCl (pH 7.5 at 298 K), 75 m*M* NaCl with 15% PEG 4K, 50 m*M* Tris–HCl (pH 7.5 at 298 K), 0.2 *M* KBr (condition No. 10 of Clear Strategy Screen I from Molecular Dimensions) and 14% 2-methyl-2,4-pentanediol, 0.25 *M* $MgCl_2$ in a 4:1:1 volume ratio; the well solution consisted of 40% PEG 8K, 50 m*M* Tris–HCl (pH 7.5 at 298 K). Crystals appeared after 3 d and grew to maximum dimensions of 0.1 × 0.05 × 0.02 mm. Before freezing in liquid nitrogen, the crystals were transferred to a solution composed of 30% PEG 4K, 50 m*M* Tris–HCl (pH 7.5 at 298 K).

2.3. Data collection and structure determination

Diffraction data for TthL1(-A) were collected from a single crystal on the BL14.2 beamline, BESSY, Berlin, Germany equipped with a fast-scanning MX-225 CCD detector from Rayonics (Evanston, USA) and a Cryojet XTL system. Data were processed and merged with the *XDS* package (Kabsch, 2010). The crystals belonged to space group $C222_1$, with unit-cell parameters $a = 63.6$, $b = 104.5$, $c = 64.3$ Å and one molecule in the asymmetric unit.

The structure was solved by molecular replacement with *Phaser* (McCoy *et al.*, 2005) using separate domains of Ser179Cys TthL1 (PDB entry 1ad2; Unge *et al.*, 1997) as a search model. The initial model was subjected to crystallographic refinement initially with *REFMAC5* (Murshudov *et al.*, 2011) and subsequently with *PHENIX* (Adams *et al.*, 2002). Manual rebuilding of the model was carried out in *Coot* (Emsley & Cowtan, 2004). As in the wild-type structure, the N-terminal residues were very flexible and could not be traced in the electron-density map. The final model of the TthL1(-A) mutant, which was refined to an R factor of 18.4% and an $R_{\rm free}$ of 22.6% at 1.95 Å resolution, includes 214 amino acids (14–157 and 159–228), 90 water molecules, one chloride ion and three PEG 4K molecules. The quality of the model was checked using *PROCHECK* (Laskowski *et al.*, 1993) and *WHAT_CHECK* (Hooft *et al.*, 1996) and showed that 92.6% of the residues have most favoured conformations, while the

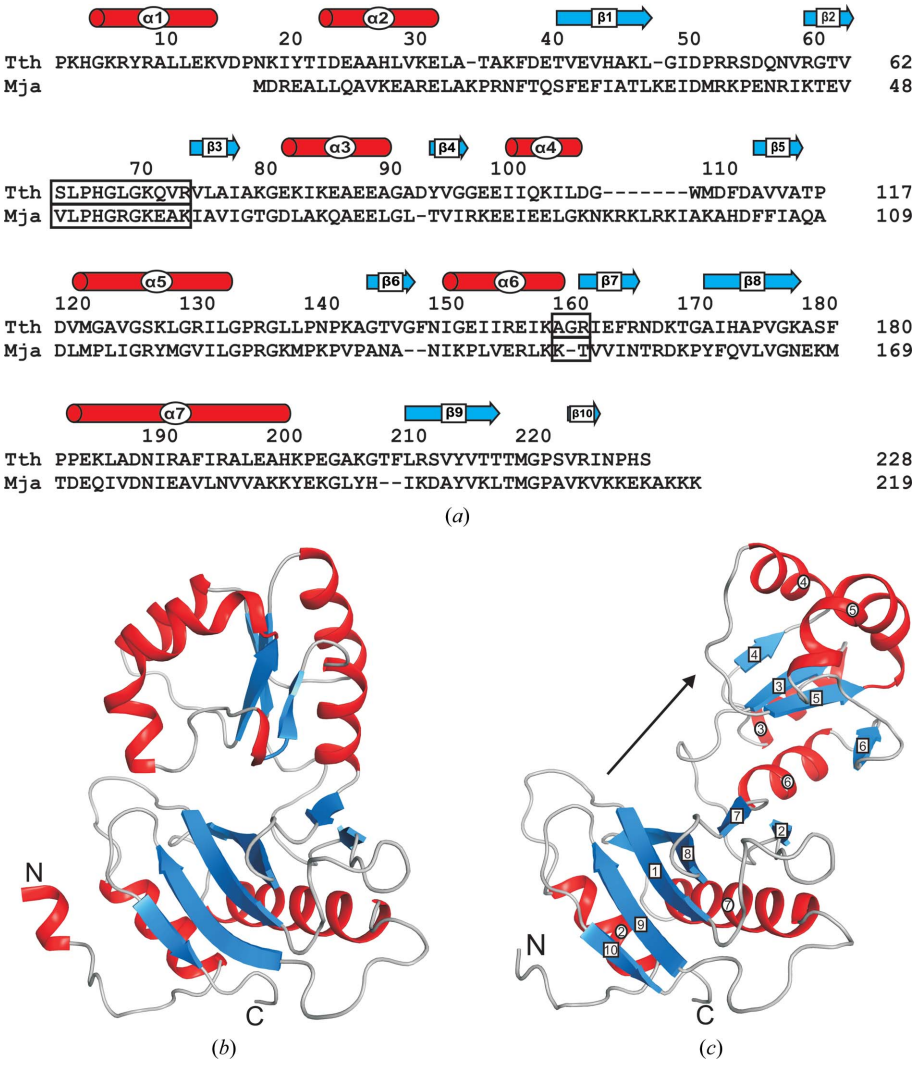

Figure 1
(*a*) Alignment of L1 amino-acid sequences from the bacterium *T. thermophilus* (TthL1) and the archaeon *M. jannaschii* (MjaL1). The numbering of residues (α-helices in red and β-strands in blue) corresponds to that of TthL1. Hinge residues are boxed. (*b*) Schematic ribbon representation of the wild-type TthL1 structure (closed conformation). (*c*) Schematic ribbon representation of the mutant TthL1(-A) structure (open conformation).

rest are in the additionally allowed region. Data and refinement statistics are summarized in Table 1. The coordinates and structure factors have been deposited in the Protein Data Bank (PDB entry 3tg8). Figures were prepared using *PyMOL* (http://www.pymol.org).

3. Results and discussion

3.1. Analysis of the L1 amino-acid sequences

Since the domains of TthL1 retain their structure in both the closed and open conformations, we supposed that their higher mobility in comparison with their archaeal counterparts could be associated with the hinge region. In all known L1 structures this region consists of two oppositely directed fragments of the polypeptide chain (Fig. 1*a*). One of these fragments is 11 residues long and connects strand β2 of domain I to strand β3 of domain II in both bacterial and archaeal L1 proteins. In the archaeal L1 proteins the oppositely directed fragment consists of two residues, one of which belongs to helix α6 and the other to strand β7. Neither of the residues is conserved. In the bacterial L1 proteins this fragment consists of three residues, one of which is a strongly conserved glycine residue, between α6 and β7.

3.2. TthL1(-A) structure description

In TthL1 the short fragment of the hinge contains Ala158, Gly159 and Arg160. To retain the flexibility of the bacterial structure that is probably caused by the glycine residue, we prepared a mutant of TthL1 lacking Ala158. This TthL1(-A) mutant was crystallized and its crystal structure was solved. The overall structure of the TthL1(-A) mutant and the notation of the secondary-structure elements are presented in Fig. 1.

Similar to other TthL1 mutants (Unge *et al.*, 1997; Nikonova *et al.*, 2007), both domains of TthL1(-A) retained their three-dimensional structure. Superposition of domains I yielded r.m.s. deviations of 0.39 Å for all C^{α} atoms excluding two loops (α2–β1, β1–β2) and of 0.26 Å for the C^{α} atoms of the antiparallel β-sheet. Superposition of domains II yielded r.m.s. deviations of 0.5 Å for all C^{α} atoms and of 0.14 Å for the C^{α} atoms of the parallel β-sheet. The only main difference in the structure of domain II was found in the orientation of the C-terminus of helix α6 joined to strand β7 of domain I. The hinge region has excellent density and is stabilized by three hydrogen bonds between its two fragments (Fig. 2). The lack of Ala158 alters the direction of helix α6 relative to the β-sheet of domain I. This causes a change in the overall shape of the molecule (Figs. 1*b* and 1*c*). As expected, shortening the α6–β7 region in TthL1 by one residue transfers the protein from the closed conformation to the open conformation, which practically coincides with that of the archaeal L1 from *Methanococcus jannaschii* (MjaL1; Fig. 3). The conformation of the short fragment of the TthL1(-A) hinge is similar to that of MjaL1 and other archaeal L1 proteins, whereas the long fragment has a significantly different conformation.

3.3. The $\alpha\beta$-corner limits the interdomain flexibility

In TthL1(-A), Gly159 is incorporated into helix α6 instead of Ala158 and consequently the helix retains its length. As a result, α6 joins to β7 without an intermediate residue, just as

occurs in the archaeal L1 proteins. The angle between strand $\beta7$ and helix $\alpha6$ determines the relative positions of the two domains. This angle is stabilized by hydrogen bonds formed by the main-chain carbonyl and amide groups of Gly159 to both $\beta7$ and $\alpha6$. All atoms making these hydrogen bonds are inaccessible to the solvent. Since no significant contacts were found between the two domains of TthL1(-A), it is possible to suggest that only these hydrogen bonds determine their mutual orientation. The two domains of TthL1(-A) are rigidly bound and can change their relative positions to only a small extent. Therefore, strand $\beta7$ and helix $\alpha6$ form a stable $\alpha\beta$-corner. A similar $\alpha\beta$-corner was also found in MjaL1 and other known archaeal L1 structures.

In wild-type TthL1, Gly159 has dihedral angles in the left-helical region of the Ramachandran plot and provides the transition from $\alpha6$ to $\beta7$. The additional residue between helix $\alpha6$ and strand $\beta7$ causes disruption of the main-chain hydrogen bond between Leu64 and Gly159 in both the closed and the open conformations. This results in the interdomain mobility. The closed and open conformations in wild-type TthL1 are stabilized by interactions between domain I and one of the protrusions (138–144 or 131–136) in domain II. In solution the bacterial L1 proteins can switch between the active open conformation, in which they are able to bind RNA, and the inactive closed conformation. Therefore, the long-linker version of L1 binds more slowly to rRNA as well as mRNA compared with the archaeal L1 version with the short linker (Nevskaya *et al.*, 2006).

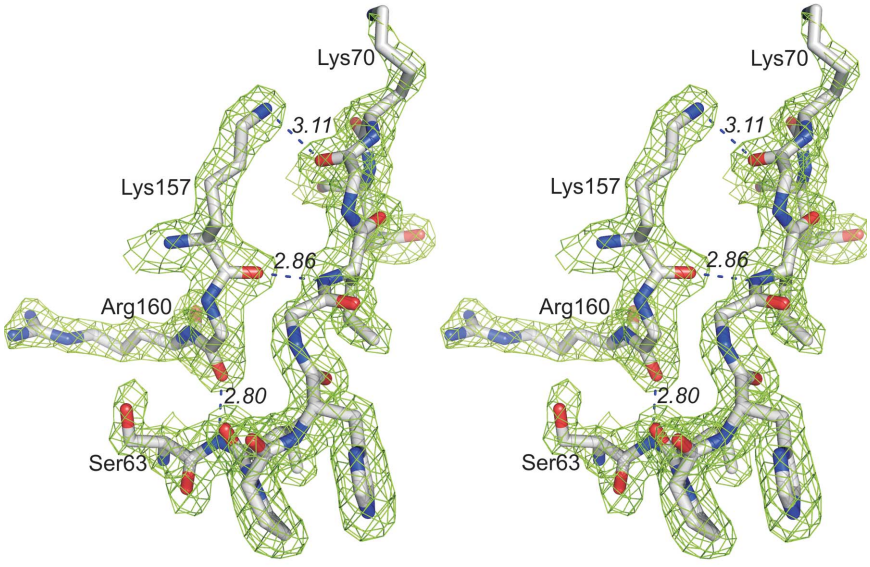

Figure 2
A stereoview showing the $2F_o - F_c$ map in the hinge region at 1.95 Å resolution contoured at 1.5σ.

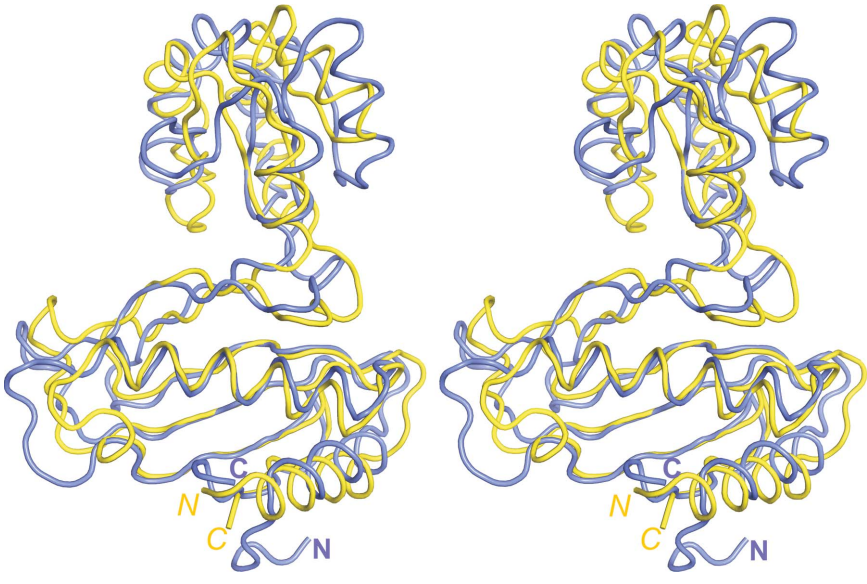

Figure 3
A superposition of TthL1(-A) (violet) and MjaL1 (yellow) with least-squares minimization of differences in C^{α} atoms of the β-sheet of domain I shows that the two structures practically coincide.

4. Conclusions

To date, the closed conformation has been found in the crystal structures of isolated ribosomal L1 proteins from two bacteria. We have now shown that this conformation emerges from the amino-acid sequence of bacterial L1 and consequently is characteristic of the structures of all bacterial L1 proteins in the isolated state. As all L1 proteins bind RNA in their open conformation, the role of the closed conformation in the isolated bacterial L1 proteins is as yet unknown. It is possible that ribosomal L1 proteins have some extra-ribosomal functions in bacterial cells. To date, one such function has been demonstrated for *E. coli* L1 (EcoL1), which displays a high level of RNA chaperone activity (Semrad *et al.*, 2004).

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