A mutant form of the ribosomal protein L1 reveals conformational flexibility

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Abstract The crystal structure of the mutant S179C of the ribosomal protein L1 from *Thermus thermophilus* has been determined at 1.9 Å resolution. The mutant molecule displays a small but significant opening of the cavity between the two domains. The domain movement seems to be facilitated by the flexibility of at least two conserved glycines. These glycines may be necessary for the larger conformational change needed for an induced fit mechanism upon binding RNA. The domain movement makes a disulfide bridge possible between the incorporated cysteines in two monomers of the mutant L1.

Key words: Ribosomal protein; X-ray crystallography; Conformational change; RNA binding; Protein synthesis

1. Introduction

The ribosomal protein L1 binds both to the ribosomal 23S RNA and to the polycistronic mRNA for L1 and L11 [1,2] which gives it ability to function as a translational repressor. L1 is not an essential protein, but viable mutants lacking L1 grow poorly and ribosomes lacking L1 show only about half the rate of protein synthesis in vitro as compared to wild-type [3]. The structure of L1 from *Thermus thermophilus* has been solved [4]. The larger of the two domains (domain I) contains both the N- and the C-terminus of the polypeptide chain and is an α/β structure. Domain II is a version of the Rossmann fold topology [5]. The domains are connected by two regions of the polypeptide in a hinge resembling fashion. The most conserved part of the structure is the surface of a narrow cavity between the two domains. The two domains make only a few non-covalent interactions at the edge of this cavity [4]. An unusual sensitivity to different chemicals, resulting in non-isomorphism between crystals, was observed during the structure determination of wild-type L1. This indicated a high degree of flexibility of the molecule and could be explained by a flexibility between the domains.

In this paper, the structure of the mutant S179C is presented. The comparison to the wild-type structure of L1 clearly demonstrates the domain flexibility.

2. Materials and methods

2.1. Preparation of T. thermophilus L1 mutant S179C

The gene encoding *T. thermophilus* L1 has been cloned and sequenced (accession no. X81375). The in vitro site-directed mutagenesis was performed essentially as described by Mårtensson et al. [6]. The preparation and overexpression of the mutant has been described [7].

2.2. Crystallization

The procedure used for crystallization was as described earlier [8] with some modifications [4]. Crystals of S179C L1 belong to space group $P2_12_12$ as the crystals of the wild-type L1 and have cell dimensions: $a = 76.05$ Å, $b = 61.42$ Å, $c = 46.11$ Å. Crystals of mutant L1 co-crystallized with sodium mersalyl heavy atom compound were used to collect diffraction data. These crystals diffracted to 1.7 Å resolution compared to about 3.0 Å for native crystals (Table 1).

2.3. X-ray data collection

Diffraction intensities were measured at the EMBL outstation on beamline BW7B of DESY, Hamburg on a large MAR imaging plate. Two data sets at different resolution and with different amount of exposure in order to measure the lower resolution data properly, were processed using the XDS program and merged with XSCALE [9]. The statistics of the data sets are listed in Table 2.

2.4. Solving the structure

The structure was solved using the molecular replacement method as implemented in the X-PLOR program [10], with the wild-type L1 model without solvent molecules, recently reported at 1.85 Å resolution [4], as a search model. After initial rigid body refinement where the two domains of the protein (domain I with residues 5–65 and 152–228, domain II with residues 66–151) were refined individually and two subsequent simulated annealing molecular dynamics simulations, electron density maps with coefficients (F_o-F_c), $(2F_o-F_c)$ and $(3F_o-2F_c)$ were calculated with X-PLOR with the free R-value file setup. Interpretation of these and rebuilding of the model were done with the program O [11]. 123 water molecules were inserted during several rounds using the automated peak search routine in MAPMAN. The co-crystallized mercury compound, three sulfate ions and one 2-methyl-2,4-pentanediol (MPD) molecule, were inserted into the refinement with harmonic potential point restraints. The refinement statistics are listed in Table 3.

Quality analysis using the program PROCHECK [12] indicated for the Ramachandran plot that 89% of the residues are in the most favored regions and no residue in a disallowed region.

Table 1
Cell parameters of some native and derivative L1 crystals

Protein	Compound	Cell parameters (Å)
S179C L1	Native	75.5×61.0×44.9
S179C L1	Zn^{2+} (co-cryst)	75.6×60.6×45.5
S179C L1	Na-mersalyl (co-cryst)	76.1×61.4×46.1
Wild-type L1	Native	82.4×63.4×44.6
Wild-type L1	$K_3UO_2F_5$ (soaking)	76.8×61.3×45.0
Wild-type L1	K_2PtCl_4 (soaking)	76.6×61.8×45.5
Wild-type L1	$KAu(CN)_2$ (soaking)	76.6×61.2×45.3
Wild-type L1	$HgCl_2$ (soaking)	76.3×61.3×44.8

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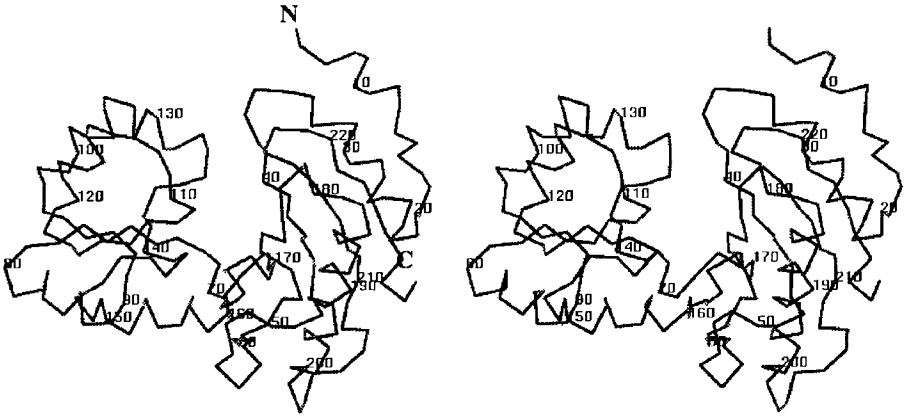

Fig. 1. A stereo view of a C_α trace of the S179C structure.

3. Results

Crystals of S179C L1 retain the $P2_12_12$ space group symmetry of crystals of the wild-type L1 previously described [4].

The structure of mutant S179C L1 was determined by molecular replacement using the two domains of wild-type L1 separately. A C_α atom trace of mutant L1 is shown in Fig. 1. The final model contains 224 amino acid residues (5–228), 123 water molecules, three sulfate ions, a MPD molecule and a mercury ion. The mercury atom of the mersalyl molecule that was included in the crystallization of the mutant has an occupancy of about 20% of a full mercury site. It binds at a region where many highly conserved residues are found such as His 172 and Glu 42. This site also binds mercury in wild-type L1 (unpublished results).

Like in the wild-type structure the N-terminal residues are very flexible and could not be traced in the electron density map. Residues 5 to 8 as well as residues 33–37 that form a loop have faint electron densities. The loop is somewhat displaced compared to its position in the wild-type structure where its electron density is well defined.

The most striking difference between the S179C mutant crystal structure compared to the wild-type crystal structure is a domain movement. A superposition of one domain in the native structure onto the equivalent domain in the mutant structure was done. The r.m.s distance differences between the main chain nitrogens, alpha carbons and carbonyl carbons of the two structures were plotted against residue number for the entire molecules (Fig. 2). This was done for both domains and the plots thus clearly indicate a domain movement in L1. The domains move away from each other by a rotation of about 7.3°, much like the opening of a clam, guided by two hinge peptides close to each other on one side of the molecule. The result is an enlargement of the cavity between the two domains in mutant L1 (Fig. 3).

The structure of each domain is essentially preserved between the wild-type and mutant L1: the mean r.m.s. distance shifts for the peptide backbone are 0.77 Å and 0.31 Å in domains I and II respectively. The large r.m.s. differences within the loop 33–37 in domain I significantly increase the mean r.m.s. value.

The links between the two domains has been suggested to serve as a hinge for the domain movements, probably necessary in an induced fit mechanism upon binding the RNA [4]. The comparison of the wild-type and the S179C structures in Fig. 3 demonstrates the presence of this hinge between the two domains. For further analysis of the region the torsion angles were plotted against sequence residue number. Focusing on the hinge regions (Fig. 4), three glycines (67, 69 and 159) clearly contribute significantly to the changes in domain orientation. These three glycines are invariable in all known bacterial sequences. A close-up plot of the r.m.s. differences in the hinge region for the two structures is shown in Fig. 2C. Again, the peptide can be seen to change its conformation at or close to the glycines in this region, in particular Gly 67 and Gly 159.

Table 2
Summary of data collection and refinement for L1 mutant S179C

Detector	MAR imaging plate,
X-ray source	DESY, EMBL outstation, Hamburg.
Wavelength of X-rays (Å)	0.87
No. of crystals	1
Space group	$P2_12_12$
Cell dimensions (Å)	$a=76.1$, $b=61.4$, $c=46.1$
Collimator aperture (mm)	0.3×0.3
Oscillation ranges	1.0° (high resolution data set)
	2.5° (low resolution data set)
Total no. of measured reflections	116835
No. of unique reflections	24181
Resolution range (Å)	12.0–1.8
Completeness (%)	98.8
Rmerge (%)	5.9
For the highest resolution shell:	
Completeness (%)	98.4
Rmerge (%)	32
I/σ(I)	8.5

Table 3
Refinement and quality of the coordinates of L1 S179C mutant

Refinement program	X-PLOR
Model building program	O
Resolution range (Å)	10–1.9
R-factor (conventional) (%)	20.3
R-free factor (%)	27.0
No. of solvent molecules	123
RMS deviations:	
bond lengths (Å)	0.013
bond angles (deg.)	1.85
dihedral angles (deg.)	25.14
impropers (deg.)	1.67

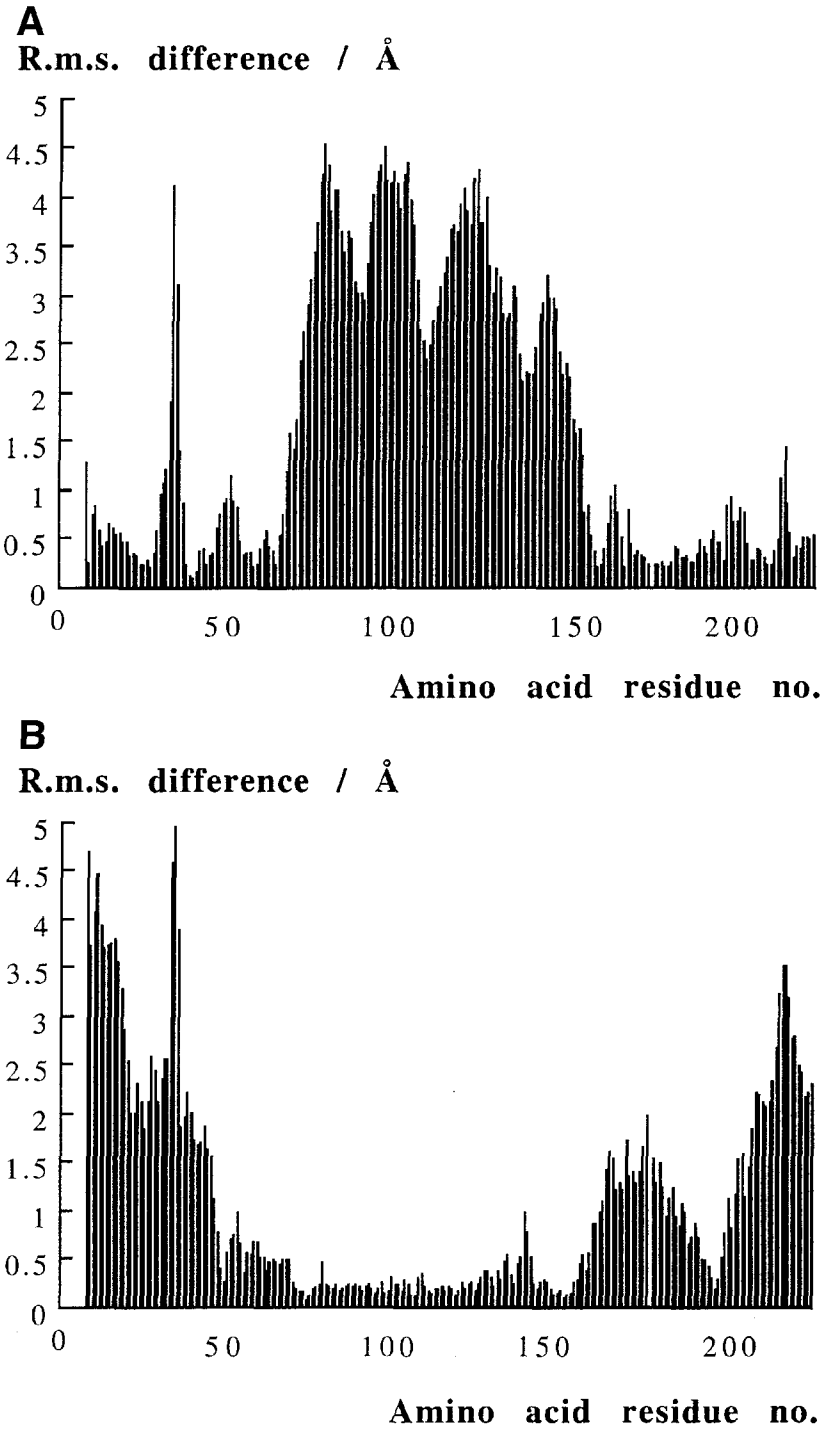

Fig. 2. Main chain root mean square differences of the S179C and wild-type crystal structures. The structures are superimposed by least-squares minimization using non-hydrogen main chain atom coordinates from domain 1 (residues 9–67 and 159–228) (A) and domain II (residues 69–158) (B) respectively. Residues 33–37 form a flexible loop with only partial density in the S179C electron density map. In the wild-type electron density map however, the loop is well defined. In (C) a close-up view of the two peptide stretches assumed to function as a hinge between the two domains.

The mutated residue is located in a short loop in domain I (Fig. 5) close to the 2-fold rotation axis in the crystals of wild-type L1 and S179C L1. In the mutant L1 crystal the cysteines from the two symmetry related molecules make a disulfide bridge. Two conformations of the bridge are present at occupancies of 50% (Fig. 6). Whether they are randomly distributed or there exists a long range ordering has not been established.

In the wild-type crystal structure the distance between the serine 179 C_α atom and its symmetry equivalent is more than 10 Å whereas in the mutant crystal the corresponding C_α atoms are less than 6 Å apart. This means for the latter

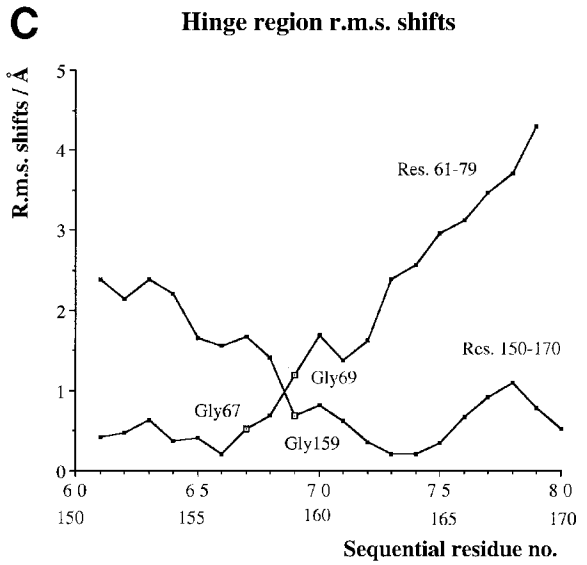

Fig. 2. (*continued*)

case that domain II would collide with its symmetry related domain of another molecule if there would be no changes in the structure, therefore the domain is forced to move away from domain I which results in the enlargement of the interdomain cavity.

The wild-type conformation of the loop of residues 33–37 could evidently not be accommodated in the S179C structure due to crystal contacts. In particular the side chain of Lys 36 would collide with both main chain and side chain atoms of Asp 111 and with main chain atoms of Phe 110 in the symmetry related molecule.

The first attempts to solve the wild-type L1 structure were complicated due to non-isomorphous derivatives (personal communication). Different mutants were made to get better binding sites for the heavy atom compounds and for one of them (S179C) crystals were obtained. When the S179C structure was solved it appeared that the mercury atom did not bind to the incorporated cysteine but in the way described above, which is the site corresponding to the mercury binding site in wild-type L1.

4. Discussion

The L1 binding site on the 23S/28S rRNA is known to be highly conserved in Bacteria, Archaea and Eukarya [13]. Accordingly, *Escherichia coli* L1 has been shown to bind specifically to a wide variety of bacterial, archaeal and even eukaryotic rRNAs [1,2,14–16]. In L1, the main contiguous region of conserved residues on the surface is found at the interface between the two domains [4]. If this region is involved in RNA recognition and binding, the cleft between the domains must be opened in order to fully display these surfaces to the RNA.

The L1 structure was found to exhibit an unusually small interdomain contact surface [4], about 250 Å^2. This is about 4% of the total area of each domain, compared to the 18–29% surface area usually buried in interdomain contacts for proteins of similar size [17]. The interdomain contacts present were also found to be few and weak. Altogether these indications make a domain movement to be a likely event and part of the mechanism for RNA binding.

The opening of the cleft in L1 observed here is probably not sufficient for the binding to double stranded RNA. The glycines offer less restricted rotations in the phi and psi angles. Several bonds in the hinge regions including glycines 67 and

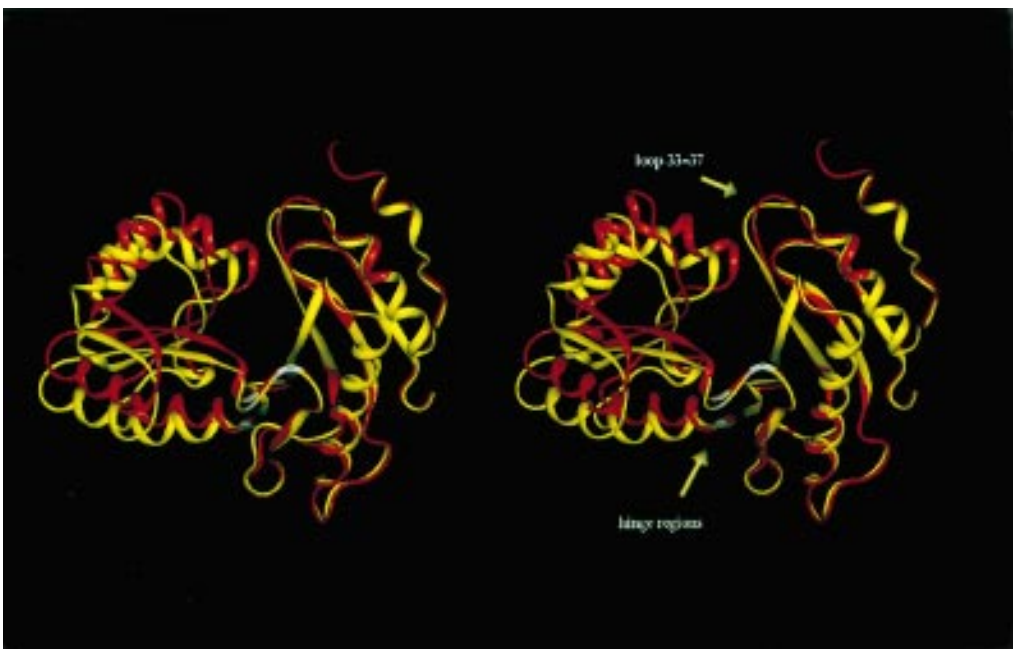

Fig. 3. A ribbon representation of the wild-type structure (red) superimposed onto the S179C structure (yellow) from least-squares minimization superposition of main chain atom coordinates of domains I. The mutant structure domain II is clearly shifted from the corresponding wild-type domain crystal structure position. The result is a more 'open' conformation where the hole between the two domains is expanded. (Residues 5–8 in the S179C structure are not indicated and were not part of the root mean square calculations for this superposition.)

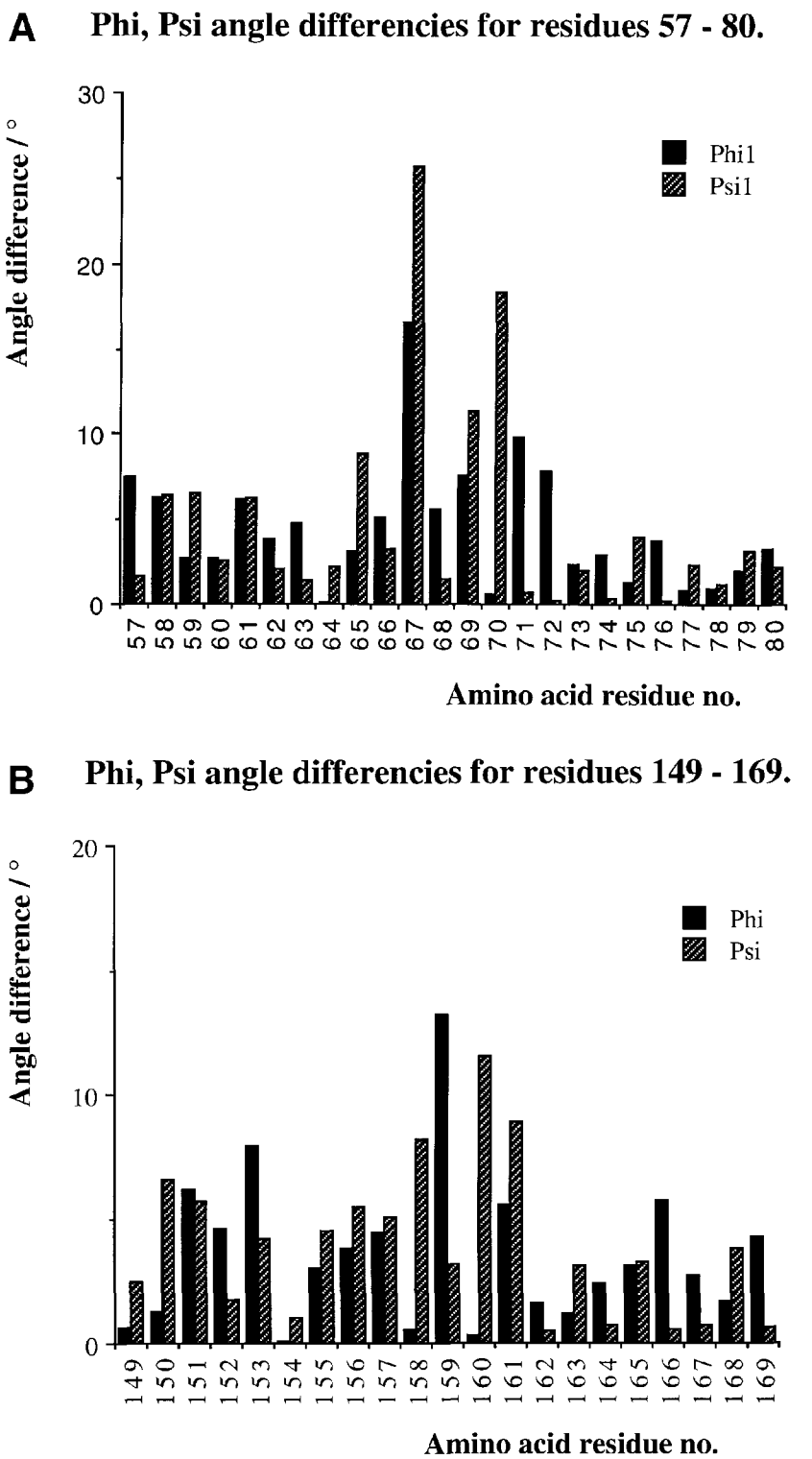

Fig. 4. A plot of the main chain phi and psi torsion angle differences between the S179C and the wild-type crystal structures in the vicinity of the hinge region. The three glycines conserved in all bacterial L1 (residues 67, 69 and 159) display some of the largest differences and might indicate an even greater importance for larger domain movements.

159 would be relatively easily rotated. Thus, in the structural context of these regions, a much larger opening of the molecule by a domain movement seems possible.

Despite the fact that the interdomain cleft is more open in the mutant the cell dimensions decrease due to closer crystal packing of the molecules with a corresponding decrease of the unit cell volume of about 8%. The sensitivity of wild-type L1 to different chemicals is shown in Table 1. Cell parameters similar to those of the mutant crystals have been observed for several different heavy atom derivatives of wild-type L1.

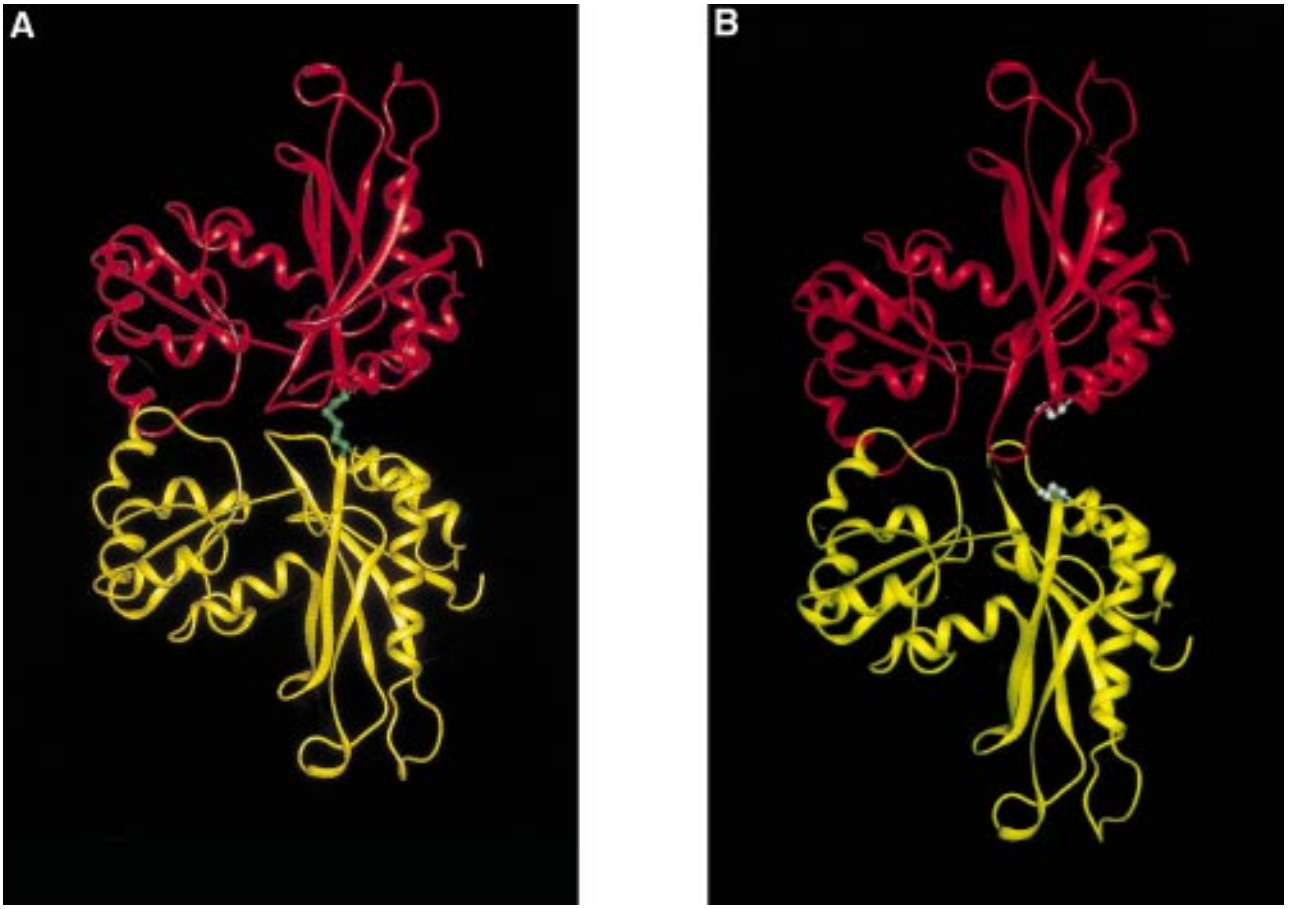

Fig. 5. The two molecules related by the two-fold rotation axis (in different colors) in the crystal is shown for the mutant (A) and the wild-type (B). The 2-fold axis (lying in the plane of the paper) is in the S179C structure not a proper one with respect to the disulfide bridge. The cysteine residues are represented in green and the two serine residues in white. The orientation of the yellow molecule is the same as in Fig. 3 apart from a small rotation around a horizontal axis. The loop in domain I (residues 33–37) that faces the corresponding loop in the symmetry related molecule can not have the same orientation as in the wild-type L1.

This may indicate the ease of which the described domain movement occurs.

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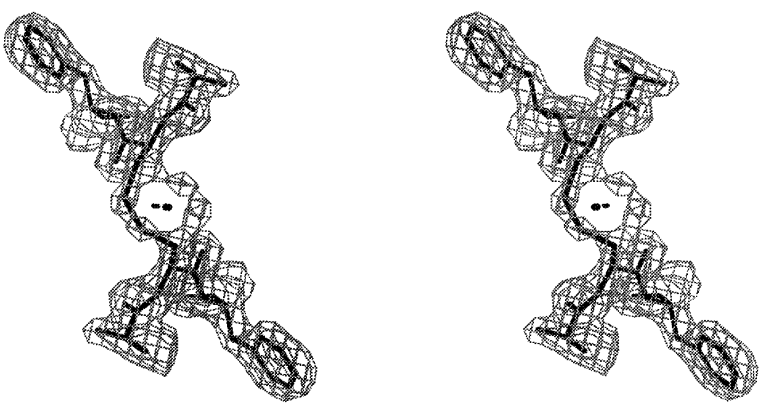

Fig. 6. A stereo view of the electron density of the region including the disulfide bridge. The 2-fold symmetry axis is indicated in the center.

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