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The length of the interdomain region of the L7/L12 protein is important for its function

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Several mutated L7/L12 proteins with changed interdomain regions were obtained. The results showed that the flexible region comprising the 39–52 amino acid residues is functionally important. Its length, but not its amino acid composition, is crucial for the function.

L7/L12 protein; Mutagenesis; Ribosomal function

1. INTRODUCTION

The acidic L7/L12 protein (120 amino acids) and its analog are found in ribosomes of all organisms and play an important role in protein biosynthesis [1,2]. The depletion of L7/L12 from ribosomes inhibits protein synthesis and decreases elongation factor activity [3–7]. Two known mutations in the N-terminal part of L7/L12 influence the level of misreading [8–10]. Structural studies of L7/L12 have shown that it contains two domains. The N-terminal part is important for L7/L12 dimerization and its binding to the ribosomes [7,12]. The C-terminal domain is globular [1,13,14]. The interdomain region includes the amino acid residues from 38 to 52 and is flexible [15–17]. The conformational changes in the L7/L12 depend on ribosomal function [18,19].

Earlier it was shown that ribosomes with a shortened interdomain region of L7/L12 decrease the translation rate in vitro [9,20] and the protein with deleted interdomain region is not active [20].

In the present communication we describe some properties of the L7/L12 with an artificial and increased in length interdomain region.

2. MATERIALS AND METHODS

The *E. coli* strain XL1 [21] was used for expression of the L7/L12 genes. All DNA manipulations and all cell growth were carried out according to a published manual [22].

The kanamycin resistance Geneblock (Pharmacia, Sweden) with symmetrical polylinker sites of its 5′- and 3′-ends was used for mutagenesis. In one case the block was ligated into the *Eco*RI site of the pPR1 plasmid [20] and the new plasmid was designated as pPR2. In another case the polylinker *Sal*I site of the plasmid pPR28 [20] was destroyed by the filling-in procedure with Klenow DNA polymerase and the Geneblock was again ligated into the *Eco*RI site, in this case creating the plasmid pPR36.

The pPR2 plasmid was digested with *Bam*HI or *Pst*I thus excising the Geneblock and, after ligation, plasmid pPR11 (with inserted codons for six amino acids) or pPR12 (with insertion of fourteen amino acids) were obtained, respectively. Similarly, the Geneblock from plasmid pPR36 was excised with *Sal*I or *Pst*I and plasmids pPR37 and pPR38 were constructed.

The cells were grown in LB broth. Induction with isopropylthiogalactoside (IPTG, 0.004%) was done at A_{590} = 0.6–0.8 o.u.

The proteins were isolated by the successive use of ion-exchange chromatography on DE-Sepharose CL-6B (Pharmacia, Sweden) with a 0–0.25 M linear sodium chloride gradient, ultrafiltration on Ultrogel AcA44 (LKB), and by FPLC on a MonoQ-column (Pharmacia, Sweden) with a 0–0.25 M linear sodium chloride gradient in 40 mM Tris-HCl (pH 7.8) buffer according to the manufacturer's manual.

Analytical centrifugation was carried out according to the method of Yphantis [23]. Heat denaturation was done as described in [24]. SDS-electrophoresis was carried out according to the published procedure [25] and cell-free translation as in [20].

Miscoding was checked at 30°C in 0.35 ml of 0.04 M Tris-HCl (pH 7.6), 0.1 M NH_4Cl, 0.01 M $MgCl_2$, 0.001 M DTT buffer with 40 pmol of 30S and 50S subunits, 0.1 mg poly-U, 0.2 mg of cell extract, 5 nmol tRNA, 0.9 nmol [^{14}C]Phe (9,100 dpm/pmol; Amersham, England), 0.9 nmol [^{3}H]Leu (3,400 dpm/pmol; Amersham, England), 0.12 μmol GTP and 0.72 μmol ATP.

3. RESULTS AND DISCUSSION

After mutagenesis four mutated proteins were obtained (Fig. 1). L12-pPR11 and L12-pPR12 are proteins with insertions of six and fourteen additional amino acids, respectively. L12-pPR37 contains ten new amino acids and L12-pPR38 has fourteen new residues (Fig. 1) instead of 39–52 residues.

All the proteins were synthesized in vivo in a high quantity (Fig. 2). Two of them, with the longest insertion and longest substitution (L12-pPR38) were purified (Fig. 2, lanes 10 and 11).

The data on poly-Phe synthesis shows that replacement of the natural interdomain region by an arbitrary

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                  39                  52
L12 w.t.          V-A-V-A-A-G-P-V-E-A-A-E-E-K-T
L12-pPR11         V-A-V-A-A-G-P-V-E-A-A-E-E-K-T-E-F-G-S-G
L12-pPR12         V-A-V-A-A-G-P-V-E-A-A-E-E-K-T-E-F-P-G-S-V-D-L-Q-V-D-G-S-G
L12-pPR37         E-F-P-G-S-V-D-G-S-G
L12-pPR38         E-F-P-G-S-V-D-L-Q-V-D-G-S-G
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Fig. 1. Amino acid sequence of the interdomain region (39–52) of the mutated and wild type L7/L12 proteins. The flexible regions of the w.t. protein are in bold letters.

amino acid sequence of the equal length (Fig. 3, pPR38) virtually does not influence the protein activity, though the protein with deleted 38–52 residues [20] are not active (Fig. 3, pPR28). The protein with the two-fold increased length of interdomain region is much less active than the wild type protein (Fig. 3, pPR12).

The experiments on miscoding show that the protein with the inserted fourteen residues (pPR12, Table I) has a lower level of miscoding. The partial deletion of the flexible region of the L7/L12 also affects the miscoding level [9,10,20].

Heat-denaturation experiments show that these proteins have a distinct melting temperature and enthalpy of denaturation. Their stability and cooperativity is different, the effective enthalpy differs from the calorimetric and their ratios vary (Table II).

It follows from the sedimentation measurements that the proteins are dimers (Table I). Earlier data demonstrate that mutated proteins replace about 50% of the wild type proteins in the ribosomes during expression in vivo [20]. All these facts indicate that the main structural features (the dimerization capacity and the C-terminal globule) are not disrupted. Thus, the functional properties of the mutated proteins can be connected with changes in the interdomain region.

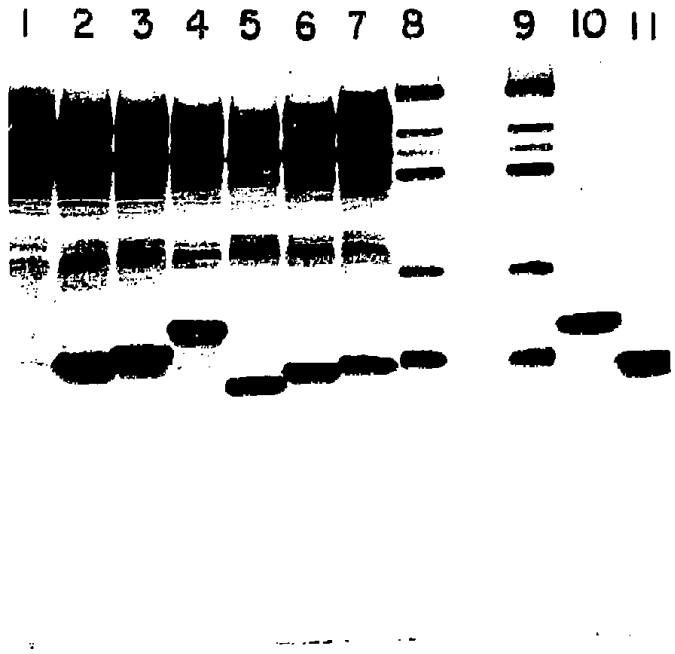

Fig. 2. SDS-electrophoresis of the cell extract and purified proteins in 15% gel. Expression of the L12 w.t. gene without induction (1) and after induction by IPTG (2); expression of L12-pPR11 (3), L12-pPR12 (4), L12-pPR28 (5), L12-pPR37 (6) and L12-pPR38 (7); markers (Pharmacia) 14, 20, 30, 43, 67, and 94 kDa (8,9); purified proteins L12-pPR12 (10) and L12-pPR38 (11).

The deletion of interdomain amino acids has a more pronounced effect than insertion. The replacement of these acids by an arbitrary sequence virtually does not

Table I

Some properties of mutated L7/L12 proteins

Protein	Miscoding		[^{14}C]Phe/[^{3}H]Leu ratio	M.wt., equilibrium sedimentation (kDa)
	[^{14}C]poly-Phe synthesis	[^{3}H]poly-Leu synthesis		
	(pmol in 10 min, 30°C)			
w.t.	120	12.1	9.9	26.5
L12-pPR12	70.2	5.9	11.9	28.0
L12-pPR38	104.8	11.5	9.1	27.3

Table II

Heat-denaturation parameters of mutated L7/L12 proteins

Protein	Temperature of denaturation (°C)	ΔH^{cal} (kcal/mol)	ΔH^{eff}	$\Delta H^{cal}/\Delta H^{eff}$ ratio	Concentration (mg/ml)
L7/L12, w.t.	69.0	97.0	41	2.4	3.05
L12-pPR28*	68.3	87.0	46	1.9	2.72
L12-pPR18*	58.0	73.5	53	1.4	2.92
L12-pPR12	64.0	80.5	43	1.9	2.80

*The protein with deleted 38–52 residues [20].
*The protein with the 44–52 deletion [20].

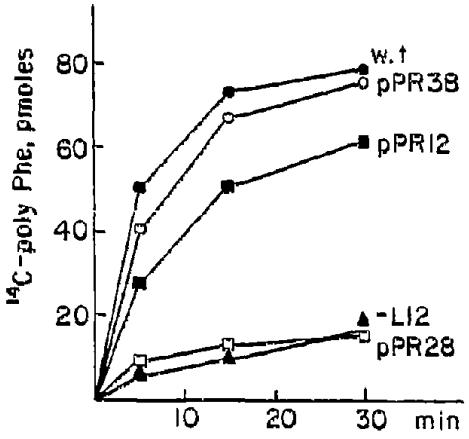

Fig. 3. Polyphenylalanine synthesis with 50S subunits (20 pmol) after their reconstruction with the shown L7/L12 proteins.

affect the function of the L7/L12. The partial deletion of amino acids adjacent to the globular part of L7 (Δ44–52) has a greater effect than the deletion of the 38–46 residues [20]. Considering all the above data, it can be assumed that the length of the interdomain region of the L7/L12 protein, but not the amino acid sequence or composition, has a crucial effect on ribosomal function.

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