Minireview

The L7/L12 ribosomal domain of the ribosome: structural and functional studies

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Abstract The L7/L12 protein forms a functionally important domain in the ribosome. This domain is involved in interaction with translation factors during protein biosynthesis. The tertiary and quaternary structure of the L7/L12 protein was established as a result of intensive studies in solution and in the ribosome. The conformational changes of L7/L12, the elongation factors Tu and G and other ribosomal proteins were traced by different experimental techniques. These changes occur upon interaction of the ribosome with the elongation factors and depend on GTP hydrolysis in accordance with the functional states of the ribosome.

Key words: Ribosome; Protein L7/L12; Structure and function; Elongation factor G; Elongation factor Tu

1. Introduction

E. coli ribosomes are the most studied protein-synthesizing ribonucleoprotein complexes (for review see [1]). It is difficult to attribute a specific and precise functional role to any individual ribosomal component in such a complicated assembly. An exception is the L7/L12 protein. It forms a well defined domain in the ribosomes. This domain is involved in interactions with translation factors during protein biosynthesis and many conformational changes take place in the ribosomal components in this case.

L7/L12 was one of the first proteins isolated from 50S ribosomal particles [2]. It was named L7/L12 protein according to Berlin's nomenclature [3,4]. This protein is present in two forms: L12 has a free N-terminus while L7 has aminoacylated N-terminal serine. Both proteins are acidic but they differ slightly in their isoelectric points [5]. Because of the close similarity they are often referred to in the literature as the L7/L12 protein. It is now commonly accepted that there are four copies of L7/L12 per ribosome [6,7].

Similar acidic proteins are found in the large ribosomal subunits of archaea [8], eukaryotes [9] and all eubacteria. Archaebacterial and eukaryotic proteins are homologous to each other, but show little homology to eubacterial L7/L12 proteins, though they are related to eubacterial protein by different physical and functional criteria [10].

The L7/L12 protein forms a clearly defined morphological feature in the *E. coli* 50S ribosomal subunit which is called the L7/L12 stalk or protuberance [11]. The outcome of intensive physico-chemical and functional studies of L7/L12 in solution and in ribosomes is the subject of the present minireview.

2. Structural studies of the L7/L12 protein

2.1. Primary structure

L7 and L12 comprise 120 amino acids each, have a molecular mass of 12 200 Da and isoelectric points of 4.70 and 4.85, respectively [12]. The protein lacks tyrosine, tryptophan, histidine, and cysteine, and contains about 25% of alanine. There are three methionine residues in positions 14, 17 and 26.

2.2. Aggregate states in solution

Sedimentation equilibrium experiments in solution without denaturing agents give a molecular mass value of about 24 kDa, however in denaturing conditions it is about 12 kDa [13]. The dimer state of L7/L12 in conventional solutions was confirmed later by high speed sedimentation. The molecular mass calculated from sedimentation ($s = 1.57$) and diffusion ($D = 5.5$) coefficients was estimated to be about 24 kDa [14,15]. Stable dimers and four copies of L7/L12 in the ribosome suggest that further association could exist with other ribosomal components. It was shown that protein L8 is a complex of L7/L12 with the L10 protein [16]. This complex can be formed in vitro and its stoichiometry is 4 mol (two dimers) of L7/L12 to 1 mol of L10 [17,18]. Oxidation of methionine residues in L7/L12 with hydrogen peroxide [14,15] causes disruption of the protein dimer and leads to its monomer state in solution. The monomer does not interact with the L10 protein.

2.3. Secondary and tertiary structure

The circular dichroism spectra revealed a high content of the α-helical structure (55–60%) and about 20% of the β-structure in the L7/L12 dimer [15,19,20]. These results are in general agreement with theoretical evaluations of the secondary structure in the protein [21,22].

Oxidized L7/L12 and its 27–120 fragment have 40–45% of the residues in the helical conformation [14,15] and do not form dimers, but the fragment 1–73 is capable of dimerizing [15] and binding to the ribosome [23]. These data have shown the essential role of the N-terminal sequence for L7/L12 dimerization.

Thermal denaturation studies of the L7/L12 dimer, oxidized (monomer) protein and that of the 27–120 fragment revealed heat absorption peaks in all these samples. The coincidence between the enthalpy values for the oxidized protein and its 27–120 fragment suggested a globular conformation of the L7/L12 C-terminal part. It follows from proton magnetic reso-

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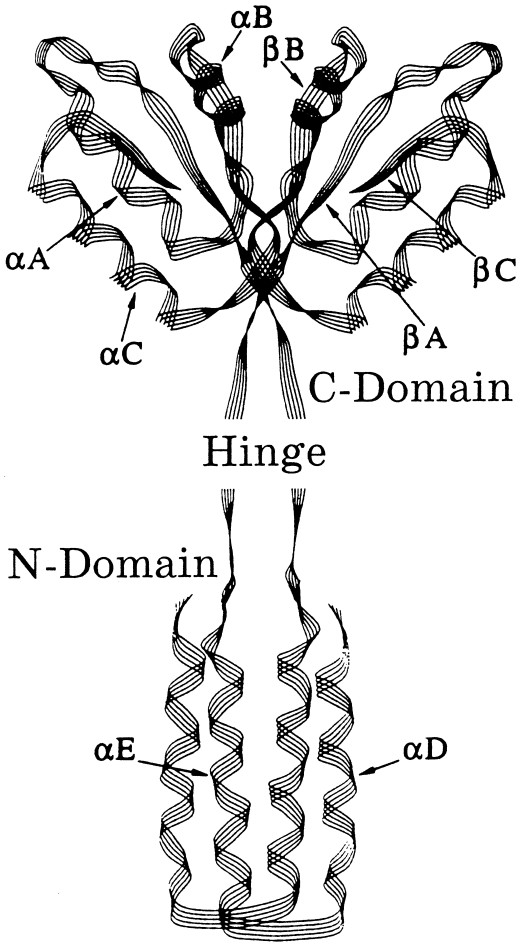

Fig. 1. Topology of secondary structure elements of the L7/L12 dimer [37].

nance data that the Phe-54 residue must be in an internal hydrophobic environment within C-terminal structure, while the Phe-30 residue participates in protein dimerization [24]. The relatively high radius of gyration indicates an elongated shape of the dimer [25,26]. On the basis of these data a model of the spatial structure of the dimer was suggested [14], where the N-terminal sequence takes part in the L7/L12 dimer formation and the C-terminal (50–120 residues) parts are globular. The tertiary structure of the C-terminal part (for residues 53–120) was determined at a resolution of 2.6 Å [27] and later refined at 1.7 Å [28].

Various arrangements of the subunits in the L7/L12 dimer have been proposed, antiparallel (head-to-tail orientation) [13] and parallel [29]. Additional details of the structure of the L7/L12 protein were obtained by NMR studies. It was shown that identical amino acids in N-terminal sequences have equivalent environments. Consequently, the N-terminal structure of the L7/L12 dimer is a symmetrical one [30].

Physical studies of L7/L12 in situ have shown that some segment of its sequence has a highly flexible structural element [31,32]. The NMR resonance signals from the L7/L12 globules are clearly seen in the spectra of the 70S ribosomes, 50S subunits and in the L7/L12-L10 complex [31,33]. The size and location of this flexible region (hinge) was determined by the NMR method. The hinge is comprised of residues 37–50 [34].

More details on the N-terminal structure were revealed by site-directed mutagenesis [35,36]. Oxidation of L7/L12 with Val-38/Cys-38 mutation occurs very easily with air oxygen or with 5′,5′-dithiobis(2-nitrobenzoic acid), and the Cys-38 mutant with the disulfide bond is active in reconstituted 70S ribosomes [36]. This suggests a parallel (head-to-head) orientation of monomers in the L7/L12 dimer. Recent NMR studies established the spatial arrangement of all the secondary structure elements of the L7/L12 dimer [37] (Fig. 1).

From the data cited above, the tertiary structure model of the L7/L12 dimer can be described as a dimeric protein with three domains, where the N-terminal parts of both monomers comprising residues 1–37 form a four-helix bundle. The C-terminal domain is formed by two associated globules, each including residues 50–120. The N- and C-terminal domains are connected with flexible strings of residues 38–49.

3. Functional studies

The site that stimulates G factor-dependent GTP hydrolysis is located on the 50S subunit [38]. Ribosomes depleted of L7/L12 [39,40] have a decreased ability to interact with the elongation factors. Reconstitution of the G factor-induced GTPase can be done after readdition of L7/L12. Selective depletion of L7/L12 from the 50S subunits suggests that the protein is not strongly bound to the ribosome. The L7/L12 protein is also essential for interaction with the initiation [41,42] and termination [43] factors. The fragment 1–73 of the L7/L12 protein prevents binding of intact L7/L12 to the ribosomes and blocks the in vitro translation [23,44]. Thus, the N-terminal part of the protein is not only responsible for its dimerization (see Section 2.3), but contributes also to its interaction with the ribosome.

A study of spin-labelled ribosomes indicated high mobility of some part of L7/L12 [45]. This fact was supported by fluorescence experiments and the data suggested that ribosome-bound tRNA can cause structural changes in the ribosome [46]. NMR studies of the protein in situ have shown that the C-terminal domain has somewhat independent mobility [31]. The flexible region 38–50 (see Section 2.3) provides the means for such mobility [34,47]. This unique property of L7/L12 is functionally important. The mobility is inhibited by the binding of elongation factor G to the 70S ribosome [48].

The functional importance of the flexible region (hinge) was further studied by site-directed mutagenesis [49–51]. All the mutated proteins were expressed from plasmids and the ribosomes isolated from cells contain mutated L7/L12: this indicates that the mutated proteins can bind to the ribosome.

The deletion of amino acids from the hinge has a more pronounced effect than their insertion [50]. Reconstitution of 50S subunits deprived of wild type L7/L12 with deletion mutants Δ44–52 and Δ38–52 produce virtually inactive ribosomes, while the insertion mutant (with 14 added amino acids)

Table 1
Trypsin sensitivity of the L7/L12 protein in ribosomal complexes with elongation factors

Ribosomes with	L7/L12 cleavage
aa-tRNA, Tu, GMPPNP	no
aa-tRNA, Tu, GDP, kirromycin	yes
G, GMPPNP	yes
G, GDP, fusidic acid	no

give functionally active subunits. On the other hand, the activity of L7/L12 with a substituted hinge region containing an arbitrary amino acid sequence does not differ from the wild type L7/L12 [50]. From these data, it was concluded that the length of the interdomain region, but not its amino acids composition, has a crucial effect on the protein function in ribosomes.

Since elongation factor G blocks mobility of the L7/L12 C-terminal domain in ribosomes, conformational changes can occur in this complex. Limited proteolysis was used as a tool to follow conformational changes in the ribosomes upon their interaction with the elongation factors.

Two main states can be distinguished at interaction of the G-factor with ribosomes: before and after GTP-hydrolysis. The state prior to GTP hydrolysis can be modelled with an uncleavable analogue of GTP and after such hydrolysis this can be done with GTP and fusidic acid [52]. In both cases the G factor is bound to the ribosome (for details see [53]). Two equivalent states exist for the ribosomal complex with elongation factor Tu [54]. It was found that the L7/L12 protein is digested in ribosomes in the pre-GTP-hydrolysis state, whereas L7/L12 is trypsin-resistant in the ribosomal complex in the post-GTP-hydrolysis state [55]. It is noteworthy that the accessibility of L7/L12 to proteolysis is reversed in the ribosome with elongation factor Tu (Table 1). This change of the L7/L12 sensitivity to trypsin correlates with the consecutive binding of elongation factors to the ribosome [56]. Factor Tu binds to the ribosome, leaving L7/L12 protease-resistant as in free ribosomes. After Tu-dependent GTP hydrolysis L7/L12 changes its conformation and is sensitive to digestion. L7/L12 is accessible to trypsin after factor G binding, i.e. before G-dependent hydrolysis of GTP. After such hydrolysis the protein conformation is restored, L7/L12 becomes trypsin-resistant, and the ribosome is ready for a new cycle.

These data suggest that conformational changes in the L7/L12 protein are induced upon interaction of elongation factors with ribosomes and that such changes occur depending on the functional states of the ribosome. At the same time, the accessibility of other proteins to proteases is also changed. Some of them, S6, S7, S15, S18, S19 and L9, are located far from the ribosomal factor-binding center [56]. The varying accessibility of these proteins to proteases cannot be explained by their direct interaction with elongation factors. On the grounds of these data it was suggested that interaction of the G and Tu factors with the L7/L12 domain (possibly including some part of the ribosomal RNA [57]) invokes structural changes in ribosomal subunits. Hence, L7/L12 forms an allosteric ribosomal domain and GTP regulates different stages of the factor-depending reactions.

The L7/L12 C-terminal globule has a conserved surface [28] which can be a functional site for proper interaction with the translation factors [23,28,58]. However, the elongation factors themselves have to bind or release the ribosome with a certain conformation. The results of the proteolysis study show that the digestion rates of the elongation factors and, apparently, their conformation depend on the nature of the bound ligands. Furthermore, the factors in the GTP conformation are more stable to proteases than in the GDP one, and their interaction with ribosomes is accompanied by changes in their structures. The conformation of the G factor after binding to the ribosome is stabilized, whereas that of the Tu factor is destabilized [59]. These facts together with the results presented in Table 1 suggest that there is a difference in the way the factors interact with L7/L12 and the ribosome. The Tu interaction leads to the ribosomal conformation which is ready for the G interaction and vice versa.

The recently solved structures of the elongation factors with different ligands have shown large conformational changes in these complexes and stimulated discussion on the molecular mechanisms of the translation process (for review see [60]). Experimental data cited in the review show that many changes occur in the ribosomal components upon interaction of the factors with the ribosome. Altogether, these achievements encourage new approaches to studying the protein synthesizing machinery.

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References

[1] Nomura M. In: Hill WE et al, editors. Ribosomes, Structure, Function and Evolution. Washington, DC: American Society of Microbiology, 1990:3–55.
[2] W. Moller, H. Castleman, Nature 215 (1967) 1293–1295.
[3] E. Kaltschmidt, H.G. Wittmann, Anal Biochem 36 (1970) 401–412.
[4] E. Kaltschmidt, H.G. Wittmann, Proc Natl Acad Sci USA 67 (1970) 1276–1282.
[5] C. Terhorst, B. Wittmann-Liebold, W. Moller, Eur J Biochem 25 (1972) 13–19.
[6] A.R. Subramanian, J Mol Biol 95 (1975) 1–8.
[7] S.J.S. Hardy, Mol Gen Genet 140 (1975) 253–279.
[8] Matheson AT, Möller W, Amons R, Yaguchi M. In: Chambliss G et al, editors. Ribosomes, Structure, Function and Genetics Baltimore, MD: University Park Press, 1980:297–332.
[9] I.G. Wool, Annu Rev Biochem 48 (1979) 719–754.
[10] A. Liljas, Int Rev Cytol 124 (1991) 103–136.
[11] W.A. Strycharz, M. Nomura, J.A. Lake, J Mol Biol 126 (1978) 123–140.
[12] C. Terhorst, W. Moller, R. Laursen, B. Wittmann-Liebold, FEBS Lett 28 (1972) 325–328.
[13] W. Möller, A. Groene, C. Terhorst, R. Amons, Eur J Biochem 25 (1972) 5–12.
[14] A.T. Gudkov, J. Behlke, N.N. Vtiurin, V.I. Lim, FEBS Lett 82 (1977) 125–129.
[15] A.T. Gudkov, J. Behlke, Eur J Biochem 90 (1978) 309–312.
[16] I. Pettersson, S.J.S. Hardy, A. Liljas, FEBS Lett 64 (1976) 135–138.
[17] A.T. Gudkov, Dokl Acad Sci USSR 230 (1976) 979–980.
[18] A.T. Gudkov, L.G. Tumanova, S.Yu. Venyaminov, N.N. Khechinashvili, FEBS Lett 93 (1978) 215–218.
[19] W. Moller, H. Castleman, C.P. Terhorst, FEBS Lett 8 (1970) 192–196.
[20] M. Dzionara, FEBS Lett 8 (1970) 197–200.
[21] O.B. Ptitsyn, A.I. Denesyuk, A.V. Finkelstein, V.I. Lim, FEBS Lett 34 (1973) 55–57.
[22] B. Wittmann-Liebold, S.M. Robinson, M. Dzionara, FEBS Lett 81 (1977) 204–213.
[23] A.J. Van Agthoven, J.A. Maasen, P.I. Schrier, W. Moller, Biochim Biophys Res Commun 64 (1975) 1184–1191.
[24] A.T. Gudkov, N.N. Khechinashvili, V.N. Bushuev, Eur J Biochem 90 (1978) 313–318.
[25] R. Österberg, B. Sjoberg, A. Liljas, I. Pettersson, FEBS Lett 66 (1976) 48–51.
[26] I.N. Serdyuk, G. Zaccai, A.S. Spirin, FEBS Lett 94 (1978) 349–352.
[27] M. Leijonmarck, S. Eriksson, A. Liljas, Nature 286 (1980) 824–826.
[28] M. Leijonmarck, A. Liljas, J Mol Biol 195 (1987) 555–580.
[29] A. Liljas, Progr Biophys Mol Biol 40 (1982) 161–228.
[30] V.N. Bushuev, N.F. Sepetov, A.T. Gudkov, FEBS Lett 178 (1984) 101–104.

[31] A.T. Gudkov, G.M. Gongadze, V.N. Bushuev, M.S. Okon, FEBS Lett 138 (1982) 229–232.
[32] I.D. Cowgill, R.G. Nichols, J.W. Kenny, P. Butler, E.M. Bradbury, R.R. Traut, J Biol Chem 259 (1984) 15257–15263.
[33] A.T. Gudkov, L.G. Tumanova, G.M. Gongadze, V.N. Bushuev, FEBS Lett 109 (1980) 34–38.
[34] V.N. Bushuev, A.T. Gudkov, A. Liljas, N.F. Sepetov, J Biol Chem 264 (1989) 4498–4505.
[35] A.V. Oleinikov, G.G. Jokhadze, R.R. Traut, Proc Natl Acad Sci USA 90 (1993) 9828–9831.
[36] A.T. Gudkov, E.V. Budovskaya, N.M. Sherstobaeva, FEBS Lett 367 (1995) 280–282.
[37] E.V. Bocharov, A.T. Gudkov, A.S. Arseniev, FEBS Lett 379 (1996) 291–294.
[38] J.W. Bodley, L. Lin, Nature 227 (1970) 60–63.
[39] K. Kischa, W. Möller, G. Stöffler, Nature New Biol 233 (1971) 62–63.
[40] E. Hamel, M. Koka, T. Nakamoto, J Biol Chem 247 (1972) 805–814.
[41] A. Kay, G. Sander, M. Grunberg-Monago, Biochem Biophys Res Commun 51 (1973) 979–986.
[42] J.L. Fakunding, R.R. Traut, J.W.B. Hershey, J Biol Chem 248 (1973) 8555–8559.
[43] N. Brot, W.P. Tate, C.T. Caskey, H. Weissbach, Proc Natl Acad Sci USA 71 (1974) 89–92.
[44] V.E. Koteliansky, S.P. Domogatsky, A.T. Gudkov, Eur J Biochem 90 (1978) 319–323.
[45] T.R. Tritton, Biochemistry 17 (1978) 3959–3964.
[46] C.G. Lee, B.D. Wells, R.H. Fairclough, C.R. Cantor, J Biol Chem 256 (1981) 49–53.
[47] A. Liljas, A.T. Gudkov, Biochimie 69 (1987) 1043–1047.
[48] G.M. Gongadze, A.T. Gudkov, V.N. Bushuev, N.F. Sepetov, Dokl Akad Nauk USSR 279 (1984) 230–232.
[49] A.T. Gudkov, M.G. Bubunenko, O.I. Gryaznova, Biochimie 73 (1991) 1387–1389.
[50] M.G. Bubunenko, S.V. Chuikov, A.T. Gudkov, FEBS Lett 313 (1992) 232–234.
[51] A.V. Oleinikov, B. Perroud, B. Wang, R.R. Traut, J Biol Chem 268 (1993) 917–922.
[52] S.W. Bodley, F.G. Zieve, L. Lin, S.T. Zieve, J Biol Chem 245 (1970) 5556–5661.
[53] Spirin AS. Ribosome Structure and Protein Biosynthesis. Menlo Park, CA: Benjamin/Cammings, 1986.
[54] F. Ecstein, M. Kettler, A. Parmeggiani, Biochem Biophys Res Commun 45 (1971) 1151–1158.
[55] A.T. Gudkov, G.M. Gongadze, FEBS Lett 176 (1984) 32–35.
[56] A.T. Gudkov, M.G. Bubunenko, Biochimie 71 (1989) 779–785.
[57] D. Moazed, J.M. Robertson, H.F. Noller, Nature 334 (1988) 362–364.
[58] Donner D, Kurland CG, Liljas A. In: FEBS 12th Meeting, Dresden, 1978. Oxford, 1979:379–385.
[59] M.G. Bubunenko, A.T. Gudkov, Biomed Sci 1 (1990) 127–132.
[60] K. Abel, F. Jurnak, Structure 4 (1996) 229–238.