FEBS Letters 367 (1995) 280–282 FEBS 15636

The first 37 residues are sufficient for dimerization of ribosomal L7/L12 protein

A.T. Gudkov*, E.V. Budovskaya, N.M. Sherstobaeva

Institute of Protein Research, Russian Academy of Sciences, 142292 Pushchino, Moscow Region, Russian Federation

Received 5 May 1995

Abstract The ribosomal protein L7/L12 with the substitution of Cys^{38} for the Val^{38} residue was obtained and studied to test the orientation of polypeptide chains in the N-terminal region of the dimer. The results show that the L7/L12 dimer has a parallel (head-to-head) orientation of subunits and that its first 37 N-terminal residues are sufficient for dimerization.

Key words: Ribosome; L7/L12 protein; Structure; Function

1. Introduction

The acidic ribosomal protein L7/L12 was found in 50S subparticles which contain four copies of L7/L12 [1,2]. It displays a clearly functional activity and is involved in all translation factor-dependent reactions of the ribosome (for review see [3]). From physico-chemical and functional studies the structural model of the L7/L12 can be described as a two domain dimeric protein, where the double-helical domain comprises the N-terminal parts of its amino acid sequence and the C-terminal (residues 52–120) parts are globules [4]. The C-terminal structure of L7/L12 was resolved at 1.7 Å resolution [5].

Oxidation of all the three methionine residues in positions 14, 17 and 26 disrupts the L7/L12 dimer structure and the oxidized protein cannot bind to the ribosome. Thus, the N-terminal part of the protein is important for its dimerization and activity [4,6,7]. According to NMR studies the C-terminal globules have a high mobility in the 70S ribosomes [8].

Various arrangements of the subunits in the L7/L12 dimer have been proposed: with antiparallel (head-to-tail [4]) and parallel orientations (reviewed in [3]). A flexible interdomain (hinge) region including 38–50 residues was established by NMR studies [9].

To test the orientations of polypeptide chains in the N-terminal region of L7/L12 and to investigate the short fragment 1–37, we studied L7/L12 with Val^{38}/Cys^{38} mutation. The data obtained show that the L7/L12-Cys^{38} protein with S-S bond can bind to the ribosome and is functionally active in reconstituted 50S subparticles. Its N-terminal 1–37 fragment prevents the binding of intact wild type L7/L12 and is a dimer.

2. Materials and methods

Plasmid pRIA7 with Val^{38}/Cys^{38} mutation was kindly provided by O. Gryaznova and gene expression was carried out in *E. coli* strain XL1. Induction with isopropylthiogalactoside (0.0004 M) was done at A_{550} = 0.6–0.8 o.u. The ribosomes from crude cell extract were precipitated and the supernatant fraction was used for L7/L12 purification.

The protein was isolated by ion-exchange chromatography on DE-cellulose using buffers with 6 M urea according to a published procedure [10]. The column dimensions were 2.6 × 14 cm and the total volume of gradient was 1.5 l.

SDS electrophoresis for intact protein was carried out as in [11], for the low molecular mass fragment as in [12], and cell-free translation was done as in [13]. Analytical centrifugation was done according to the Yphantis technique [14]. The 50S subunits depleted of L7/L12 were obtained by 50% ethanol extraction [15] and 50S subunit reconstruction with L7/L12Cys^{38} was done as in [16].

2.1. *Modification of L7/L12Cys^{38} mutant and its cleavage*

Oxidation of L7/L12Cys^{38} was done with air oxygen by stirring the solution in a magnetic stirrer, or with 1 mmol 5′,5′-dithiobis (2-nitrobenzoic acid). The protein is oxidized completely also during a storage period of over a month or two.

Modification of L7/L12Cys^{38} (after reduction with 2 mmol of DTT at 37°C) was carried out with thiocyano(2-nitrobenzoic acid) according to a published procedure [17] and cleaved at 37–40°C in sodium-borate buffer, pH 9.1 for 16 h. The fragments 1–37 and 38–120 were separated on a Sephadex 75 column (1.5 × 150 cm) in Tris-HCl buffer, pH 7.5 with 6 M of urea.

3. Results and discussion

The isolated L7/L12Cys^{38} protein is very easily oxidized with air oxygen or dithiobisnitrobenzoic acid. A covalent dimer with an S-S bond is formed (Fig. 1). The calculated molecular mass from equilibrium sedimentation experiments is 2,400 ± 2,000 daltons. This value is the same as for the dimer form. Formation of the cross-linked L7/L12 dimer does not depend on protein concentrations so it can be concluded that the S-S bond is formed in the dimer state of L7/L12.

50S subunits reconstituted with the cross-linked dimer are active in a poly(U)-translation test (Table 1). Moreover, the NMR-spectra show that L7/L12Cys^{38} with the S-S bond is very much alike the wild type protein (Fig. 2).

Considering the data cited above, we can conclude that formation of the S-S bond in the mutant protein does not influence the flexibility of the hinge region (residues 38–50), which is functionally important [18,19] and does not distort the structure of the N-terminal sequence which is responsible for protein dimerization [4,6].

Since the dimer has a symmetrical structure [20] we can assume that the cysteins are very close spatially and that the polypeptides chains have a parallel (head-to-head) orientation in the N-terminal domain of the L7/L12 dimer (Fig. 3). The protein with the substitution of Ser^{33} for Cys^{33} is easily oxidized also, but the crosslinked dimer is not functional [21].

The isolated 1–37 N-terminal fragment is capable of interacting with 50S subparticles depleted of L7/L12 and interfering with the binding of wild type intact protein (Table 1). Oxidation

*Corresponding author. Fax: (7) (095) 924 04 93.
E-mail: gudkov@sun.ipr.serpukhov.su

SSDI 0014-5793(95)00564-1

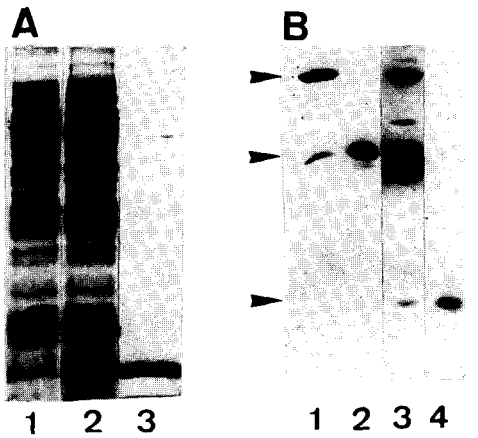

Fig. 1. SDS-electrophoresis of the crude cell extracts and purified L7/L12 protein and its derivatives. (A) 12% gel; 1, crude extract of cells without plasmid; 2, crude extract with plasmid; 3, purified L7/L12Cys^{38} protein. (B) 15% gel with 6 M urea, arrow heads indicate positions of the L7/L12Cys^{38} with the S-S bond, the L7/L12Cys^{38} protein and the 1–37 fragment from top to bottom, respectively; 1, oxidized L7/L12Cys^{38}; 2, L7/L12Cys^{38} modified by 2-nitro-5-thiocyanobezoate; 3, the protein as in slot 2 after cleavage; 4, purified 1–37 fragment.

Table 1
Poly(U)-directed polyPhe-synthesis by ribosomes containing the L7/L12Cys^{38} mutant and its N-terminal fragment

Components of the mixture	polyPhe-synthesis (%)
30S + 50S	100*
30S + 50S(−L7/L12)	20
30S + 50S(−L7/L12) + L7/L12wt	94
30S + 50S(−L7/L12) + L7/L12Cys^{38} with the S-S bond	48
30S + 50S(−L7/L12) + L7/L12Cys^{38} unoxidized	60
30S + 50S(−L7/L12) + 1–37 fragment	21
30S + 50S(−L7/L12) + 1–37 fragment + L7/L12wt	22
30S + 50S(−L7/L12) + oxidized 1–37 fragment	25
30S + 50S(−L7/L12) + oxidized 1–37 fragment + L7/L12 wt	108

*62 pmol of [^{14}C]phenylalanine incorporated into acid-insoluble polypeptide by intact ribosomes after 30 min incubation at 37°C was taken as 100%.

of the 1–37 fragment 14, 17 and 26 methionine residues with hydrogen peroxide prevents binding of the fragment to the ribosome and intact L7/L12 protein can restore 50S subparticle function. This means that 1–37 residues are sufficient for dimer formation. The NMR-spectrum also indicates the dimer state of the 1–37 fragment. Oxidation of the methionine residues leads to changes in the high- and lowfield parts of the spectrum. The fragment structure is disrupted and the signals in the spectrum became narrower (Fig. 4). The detailed structure of the L7/L12 N-terminal part remains to be solved.

Acknowledgements: We are thankful to Drs. V. Bushuev and E. Bocharov for NMR measurements. This work was supported by Grant 94-04-12051.a from the Russian Foundation for Fundamental Research.

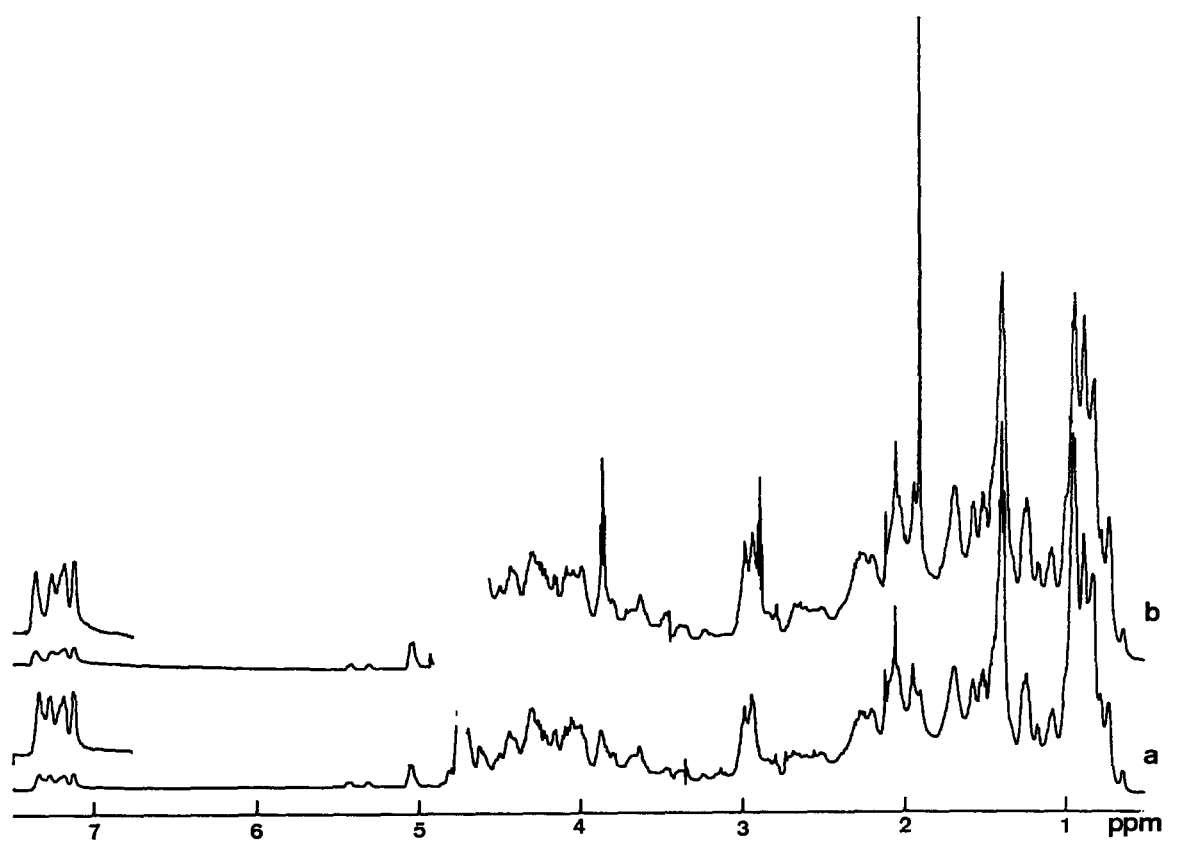

Fig. 2. 500 MHz PMR spectra of L7/L12Cys^{38} protein with the S-S bond (b) and L7/L12 wild type protein (a).

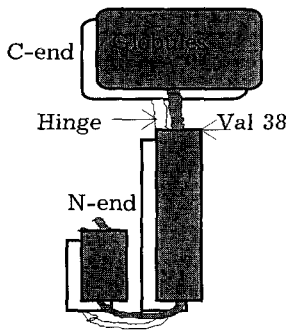

Fig. 3. Schematic model of the L7/L12 dimer. Rectangles represent secondary structure element as given in [4].

References

[1] Subramanian, A.R. (1975) J. Mol. Biol. 95, 1–8.
[2] Hardy, S.J.S. (1975) Mol. Gen. Genet. 140, 253–279.
[3] Liljas, A. (1982) Progr. Biophys. Mol. Biol. 40, 161–228.
[4] Gudkov, A.T., Behlke, J., Vtiurin, N.N. and Lim, V.I. (1977) FEBS Lett. 82, 125–129.
[5] Leijonmarck, M. and Liljas, A. (1987) J. Mol. Biol. 195, 555–580.
[6] Gudkov, A.T. and Behlke, J. (1978) Eur. J. Biochem. 90, 309–312.
[7] Calldwell, P., Luk, D.C., Weissbach, H. and Brot, N. (179) Proc. Natl. Acad. Sci. USA 75, 5349–5352.
[8] Gudkov, A.T., Gongadze, G.M., Bushuev, V.N. and Okon, M.S. (1982) FEBS Lett. 138, 229–232.
[9] Bushuev, V.N., Gudkov, A.T., Liljas, A. and Sepetov, N.F. (1989) J. Biol. Chem. 264, 4498–4515.
[10] Möller, W., Groene, A., Terhorst, C. and Amons. (1972) Eur. J. Biochem. 25, 5–12.
[11] Weber, K. and Osborn, M. (1975) in: The Proteins, Vol. 1 (Neurath, H., Hill, R. and Boeder, C. Eds.) Academic Press, New York, pp. 180–250.
[12] Schägger, H. and von Jagow, G. (1987) Anal. Biochem. 166, 368–379.
[13] Gavrilova, L.P., Kostiashkina, O.E., Koteliansky, V.E., Rutkevitch, N.M. and Spirin, A.S. (1976) J. Mol. Biol. 101, 537–552.
[14] Bowen, T.J. (1971) An Introduction to Ultracentrifugation, John Wiley, London, NY.
[15] Hamel, E., Koka, M. and Nakamoto, T. (1972) J. Biol. Chem. 247, 805–814.
[16] Gudkov, A.T., Tumanova, L.G., Gongadze, G.M. and Bushuev, V.N. (1980) FEBS Lett. 109, 34–38.
[17] Jacobson, G.R., Stark, G.R., Schaffer, M.H. and Vanaman, T.C. (1973) J. Biol. Chem. 248, 6583–6591.
[18] Gudkov, A.T., Bubunenko, M.G. and Gryaznova, O.I. (1991) Biochimie 73, 1387–1389.
[19] Bubunenko, M.G., Chuikov, S.V. and Gudkov, A.T. (1992) FEBS Lett. 313, 232–234.
[20] Bushuev, V.N., Sepetov, N.F. and Gudkov, A.T. (1984) FEBS Lett. 178, 101–104.
[21] Oleinikov, A.V., Jokhadze, G.G. and Traut, R.R. (1993) Proc. Natl. Acad. Sci. USA 90, 9828–9831.

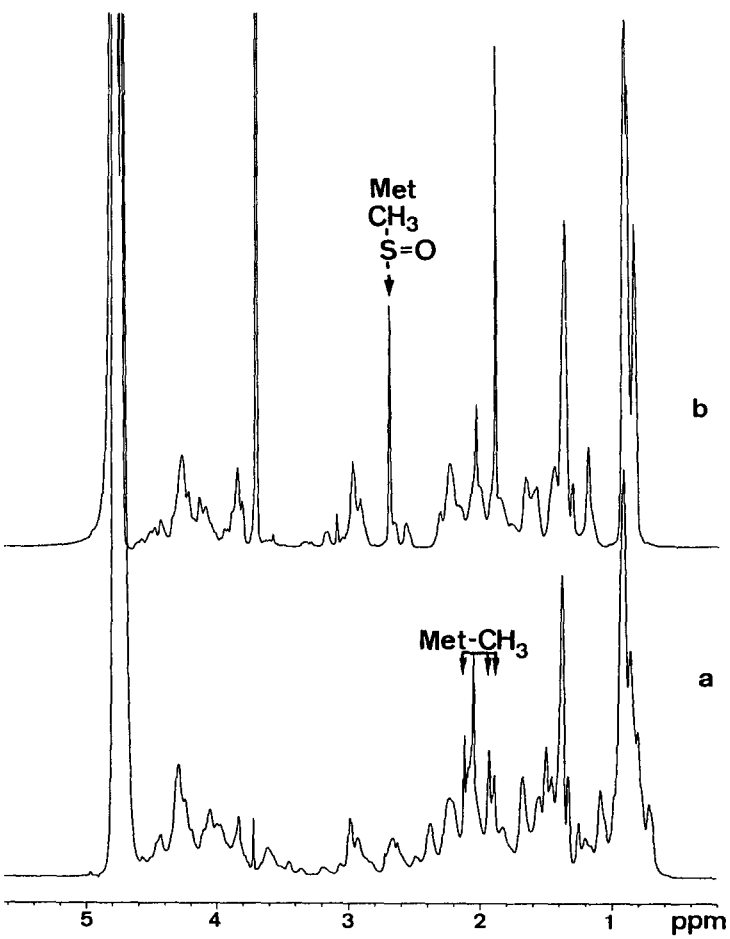

Fig. 4. 600 MHz PMR spectra of oxidized 1–37 fragment (b) and unmodified 1–37 fragment (a).