TERTIARY AND QUATERNARY STRUCTURE FOR RIBOSOMAL PROTEIN L7 IN SOLUTION

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1. Introduction

Protein L7 was the first ribosomal protein the primary structure of which was determined [1]. The high α-helical content determined experimentally [2] was in good agreement with the results of the theoretical prediction of protein L7 secondary structure [3]. The protein L7 was found to exist in solution as a dimer [4]. Small-angle X-ray scattering studies evidenced of the highly elongated shape of the dimer; the data were interpreted in terms of the model of the model of a rod 130 Å long with a diameter of 17 Å [5] or a triaxial ellipsoid with semiaxes of 90 Å, 16 Å and 6 Å [6].

The essential role of the N-terminal sequence of protein L7 for its dimerization has been shown by one of us [7].

This paper reports the result of the combined use of the recent stereochemical theory of the tertiary structure of globular proteins [8–10] and the set of experimental chemical and physical approaches [7,11] for the study of the protein L7. The tertiary and quaternary structure for the protein L7 dimer in solution is proposed. This structure can be presented as a dumb-bell where the two N-terminal helical regions interact with each other in the antiparallel manner thus forming a dimer, while the distal C-terminal parts of the subunits are globular.

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2. Materials and methods

The results of sedimentation studies of protein L7, of its 27–120 fragment and of protein L7 with oxidized methionine residues in positions 14, 17 and 26 have been reported earlier [7]. The data on infrared, CD and NMR spectra, as well as on calorimetric measurements are given elsewhere [11]. Synthetic polypeptides having amino acid sequence of the 1–26 N-terminal fragment of protein L7 [12] were also used in the experiments.

3. Results and discussion

3.1. *Experimental*

A number of important structural features of protein L7 can be deduced from several groups of experimental data available.

(i) In the native state, the protein L7 in solution is known to exist in the form of dimers [4,5,7]. At the same time, oxidation of the methionine residues in positions 14, 17 and 26 into sulphoxide, or the cleavage of the first 26 amino acids by cyanogen bromide leads to the loss of ability of the oxidized protein or the remaining 27–120 fragment to dimerize (table 1). On the grounds of these data a conclusion is made on the essential role of the 1–26 N-terminal sequence of protein L7 for its dimerization [7].

Moreover, the oxidized protein L7 cannot be a substitute for the intact protein in factor-dependent reactions on the ribosome [13]. At the same time, the fragment 1–73 of protein L7 is capable of binding with the ribosome [14]. Thus, it can be presumed that

Table 1
Some characteristics of the protein L7, the oxidized protein L7 and the fragments (1–26) and (27–120)

Sample	$s_{20,w}$	$D_{20,w}$	$\bar{v}$	$M_{S,D}$	M (equilibr.)	f/f_o	Helical content (%)	ΔH	
								kcal/mol (eff.)	kcal/mol (calor.)
L7	1.57 ± 0.1	5.5 ± 0.5	0.760[a]	24 000 ± 2000	24 400 ± 1000	1.76 ± 0.13	50–55	–	–
L7 oxidized	1.04 ± 0.04	8.3 ± 0.3	0.760[a]	12 700 ± 1000	12 500 ± 500	1.64 ± 0.05	41–43	61	60
Fragment (27–120)	0.9 ± 0.06	10.3	0.753[a]	9500[a]	9970 ± 500	1.47 ± 0.05	41–43	61	58
Fragment (1–26)			0.760[a]	2760[a]	2750 ± 200				

[a] Calculated from the amino acid composition

the ability to dimerize and to bind with the ribosome is determined by a region of the sequence in the fragment 1–73 [7].

(ii) The coincidence between the effective enthalpy of melting (calculated from the half-width of transition, see table 1) and the calorimetric enthalpy (calculated from the area under the curve) for the oxidized protein L7 and the fragment 27–120 evidences of such a structure in the C-terminal region of the protein which melts cooperatively [11]. This suggests the globular conformation of the C-terminal part of the protein L7.

(iii) The data on NMR spectra of the dimer of L7 protein, of its 27–120 fragment and of the oxidized protein indicate that the Phe^{54} residue must be in an internal hydrophobic environment within the C-terminal region structure. (The spectrum signal does not change depending on the monomeric or the dimeric state of the protein in the samples studied, while the Phe^{30} residue participates in dimerization of the protein; the signal depends on the monomeric or dimeric state of the protein in solution [11].)

(iv) The high α-helical content (see table 1) and the absence of β-structures [11] are characteristic of the protein L7 and of its dimer. This permits the assumption that dimerization is stipulated by the contact of helical regions of the two protein subunits.

(v) The high friction ratio (see table 1) and the data of X-ray diffuse scattering [5,6] evidence an elongated shape of the protein L7 dimer in solution.

3.2. *Theory*

(i) The identity of the subunits in the protein L7 dimer and the high association constant (over $10^7\ M^{-1}$) satisfy the principles of organization of subunit proteins [15,16]. It can be assumed that the dimer has a symmetry axis of the second order with an antiparallel orientation of the subunits [15,16].

(ii) There can be a helical region from residue 4–41 in the N-terminal sequence of the protein L7 [3]. In the helical net of this helix it is easily seen that side groups of hydrophobic amino acid residues form a hydrophobic strip in the region from residue 16–41 (fig.1). The contact of these two α-helices will ensure the formation of a dimer at the expense of massive and complementary hydrophobic interactions, the amino acid residues with large side groups (Val, Ile) being in contact with the smaller ones (Ala). In such

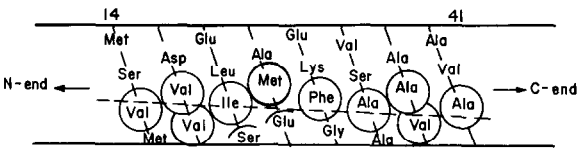

Fig.1. Two-dimensional surface of the helical region (14–41) of protein L7. Circles indicate residues contacting with the other protein molecule. Dashed line shows direction of hydrophobic strip.

an interaction, the helices are shifted relative to each other by a half-turn; their axes form a small angle (about 6°) and are antiparallel (see scheme, fig.2). The side groups of Met^{26} and Phe^{30} slightly protrude away from the region of contact. Two different areas are formed in the bi-helical structure, one with a predominance of hydrophilic residues and the other with many hydrophobic side groups. It is possible that the hydrophobic area of the dimer can serve for binding with the ribosome and/or with protein L10 [17].

(iii) A globular structure of the C-terminal part of the protein L7 molecule was constructed using the recent theory of spatial structure of proteins [8–10]. This globule is formed by two adhering hydrophobic surfaces. One of these surfaces is formed by helices 51–59 and 65–73, and the other by helices 93–101 and 105–113. There is also a helical region 80–88 and an elongated chain fragment 75–79. The helix 80–88 is located in the region of the N-termini of helices 65–73, 93–101 and the C-termini of helices 51–59 and 105–113. The elongated chain fragment 75–79 is on the surface of the globule between helices 65–73 and 105–113. There is a hydrophobic cavity on the surface

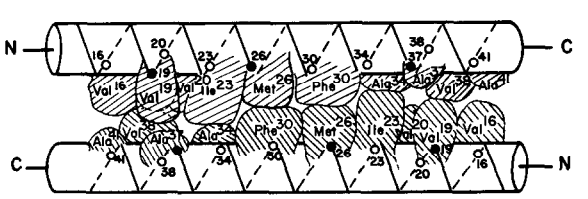

Fig.2. Scheme of the bi-helical part of the protein L7 dimer. Hatching indicates contacting amino acid side groups. Circles show C_α-atoms (filled circles above the plane of the scheme and open circles below it). Hydrophilic amino acids predominate on the reverse side of the bi-helical part (see text).

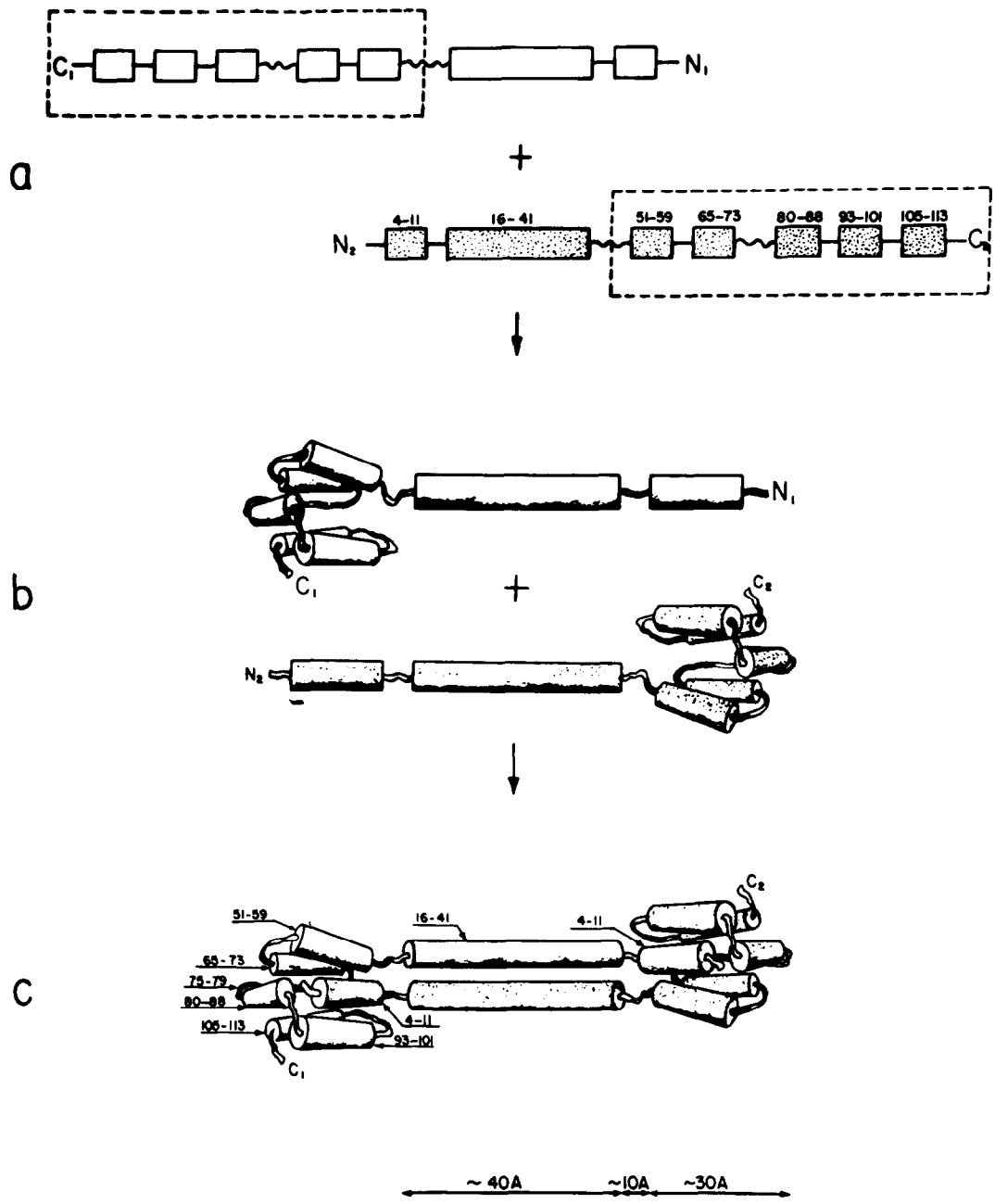

Fig.3.Scheme of the tertiary and quaternary structure formation of the protein L7 (monomer and dimer). (a) Distribution of α-helical regions in the molecule. The regions included in boxes form the globular parts of the monomers. (b) Tertiary structure of monomers. (c) Quaternary structure of the protein L7. Arrows indicate helical regions and elongated chain fragment 75–79. At dimerization of monomers, the N-terminal helix 4–11 of one molecule, fits into the globular part of the other and vice versa. Helices 16–41 of both monomers interact according to the scheme in fig.2. The three-dimensional model of the L7 dimer assembled from CPK atomic models has linear dimensions shown in the figure.

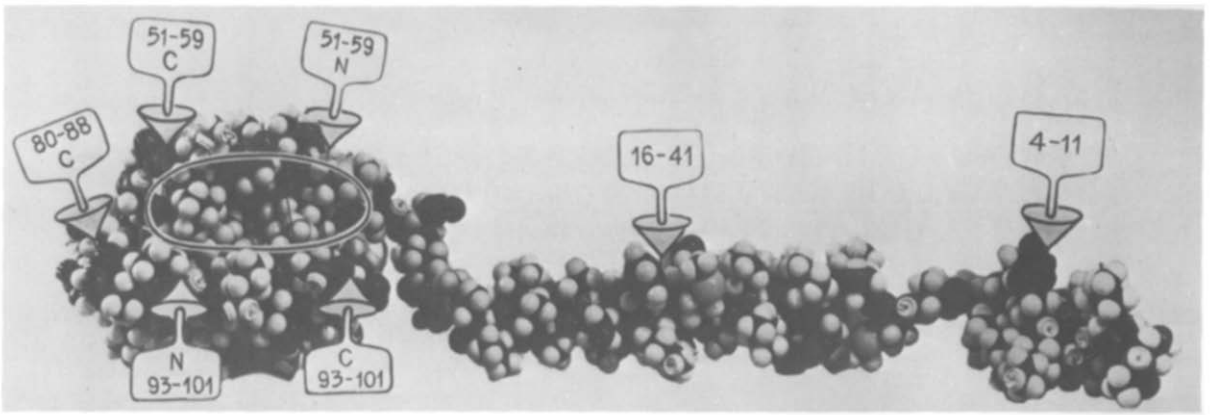

Fig.4. Photo of the atomic model of the protein L7 (monomer). Figures show helical regions. N and C indicate the corresponding helical ends. The oval between helices 51–59 and 93–101 indicates the cavity into which the helix 4–11 of another monomer fits at protein dimerization.

of this globule between the helices 51–59 and 93–101. At dimerization of protein L7, the hydrophobic surface of the helix 4–11 of one molecule will fit into the hydrophobic cavity of the other molecule and vice versa.

The scheme of the three-dimensional structure of the protein L7 monomer and dimer is seen in fig.3. A photo of the atomic model of the L7 protein in the monomeric form is given in fig.4.

The linear dimension along the long axis of the dimer in the model proposed (120 Å) is smaller than those claimed on the basis of small-angle X-ray diffuse scattering (130 Å [5] or 180 Å [6]). However, the relatively large experimental values of the radius of gyration reported in these papers (38 Å [5] and 41 Å [6]) do not contradict the model.

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