STUDY OF THE TERTIARY STRUCTURE OF PROTEIN L11 FROM *ESCHERICHIA COLI* RIBOSOMES IN SOLUTION BY PROTON MAGNETIC RESONANCE

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1. Introduction

Here we report the tertiary structure of protein L11 from the 50 S subparticle of *E. coli* ribosomes in solution. This protein plays an important role in association of ribosomal subparticles [1] and directly interacts with the 23 S RNA [2]. Protein L11 consists of 141 amino acid residues [3] and has rather high helical content (35–40%) [4].

To study structural features of protein L11 in solution we used the technique of proton magnetic resonance (PMR) which gives information on the structure of proteins and the role of their parts in the formation of this structure [5]. Earlier studies of ribosomal proteins S15, S4 and S16 performed using this method have shown that the proteins in solution have well-formed tertiary structures [6–8].

The study of the oxidized derivative of protein L11 and its N-terminal (1–81) and C-terminal (37–141) fragments has shed light on the role of the different parts of the protein sequence in the formation of the tertiary structure. The main results of this study are as follows: (1) protein L11 has a well-formed tertiary structure; (2) the N-terminal moiety of the molecule forms the most compact and stable core in the L11 globule.

2. Materials and methods

The following buffer solutions were used: A, 0.1 M Tris-HCl, pH 8.1, 1 mM β-mercaptoethanol; B, 20 mM sodium phosphate, pH 6.6, 0.3 M KCl in ^2H_2O; C, 0.1 M Tris-HCl, pH 8.0, 0.1 M KCl, 1 mM EDTA.

Isolation of protein L11 was carried out as in [4]. N-Terminal fragment 1–37 and C-terminal fragment 38–141 were obtained by cleavage of the protein at cysteine residue [10] and separated on a Sephadex G-75 column (1.5 × 160) in sodium acetate buffer with 6 M urea. The purity of the fragments was checked by amino acid analysis and SDS electrophoresis in 12.5% polyacrylamide gel [11].

Oxidation of the protein was carried out as in [11] with preliminary shielding of the thiol group by iodoacetamide.

Electrophoresis of the oxidized and intact protein L11 was carried out in a 6% polyacrylamide gel. Gel buffer, 0.1 M bis(tris) with acetic acid, pH 6.5; cathode buffer, 0.02 M bis(tris), pH was adjusted to 7.2 with morpholinoethanesulphonic acid; anode buffer, 0.03 M bis(tris) with acetic acid, pH 6.2.

Tryptic hydrolysis was carried out in buffer A at 37°C. Enzyme/substrate ratio was 1:100. The tryptic fragment was isolated on a Sephadex G-75 column (1.6 × 100) in 5% acetic acid.

Titration of the SH-group with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (Sigma) was carried out as in [12].

PMR spectra were recorded on a WH-360 spectrometer (Bruker, FRG) in the pulse mode with a following Fourier transform. The pulse duration was 6 μs (pulse angle 65°), the time of 1 cycle was 1.8 s. Chemical shifts were measured relative to sodium 2,2-dimethyl-2-silapentane sulphonate as an internal standard. The spectra were recorded in a 5 mm tube in buffer B. The proteins were introduced into the buffer from the solution with 5 M guanidine chloride by dialysis. The protein concentration was 1.5–4 mg/ml.

3. Results and discussion

3.1. *Chemical studies and tryptic hydrolysis*

Protein L11 contains a single Cys residue in position 38 [3]. Titration of this residue with DTNB under non-denaturing conditions proceeds quickly and completely. Addition of 6 M urea does not lead to a further increase of absorption at $\lambda = 412$ nm. Thus it can be concluded that the SH-group of the Cys 38 is accessible to modifying agents and is located on the protein surface.

Data of the trypsinolysis kinetics indicate the presence of a trypsin-stable fragment in the protein (fig.1). Accumulation of the fragment with M_r 8000–9000 proceeds up to 5 min which suggests a well-formed compact structure. Amino acid analysis, determination of N- and C-terminal amino acids (the N-terminal amino acid has not been revealed while Lys, Leu, Val have been found on the C-terminus) and the M_r value suggest that a fragment with the sequence 1–80 or 1–81 is formed as a result of the trypsinolysis. The conclusion on a well-formed compact structure of the N-terminal fragment is corroborated by PMR data (see below).

Oxidation of methionine residues of the protein by H_2O_2 results in a decrease of the electrophoretic mobility (fig.2a). This result can be explained by unfolding of a part of the protein polypeptide sequence of the oxidized protein (see also the PMR data). In other words, the intact protein has a more compact structure.

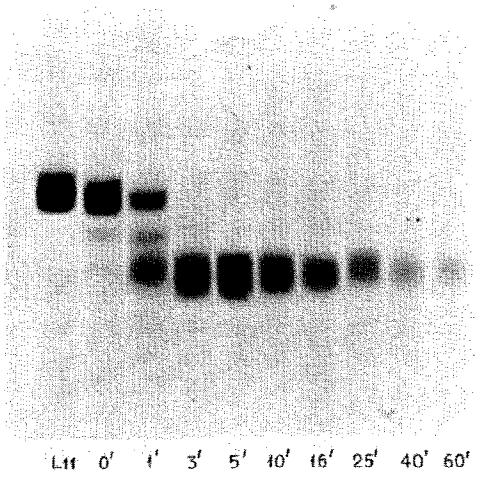

Fig.1. Kinetics of tryptic hydrolysis of protein L11 (SDS electrophoresis in a 12.5% gel). The enzyme/substrate ratio was 1:100. Tryptic hydrolysis was carried out at 37°C.

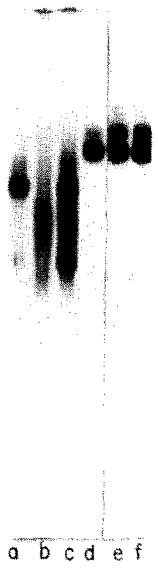

Fig.2. Electrophoresis in a 6% gel: (a) oxidized protein L11; (b) intact protein L11; (c) mixture of oxidized and intact proteins; (d–f) the same as (a–c), but in the presence of 4 M urea.

We have failed so far to choose electrophoretic conditions (without denaturating agents and within pH 6–8) under which the intact protein would give a narrow band (fig.2b). It seems that the 'smearing' of protein L11 is due to the small stability of the protein structure as a whole. Differences in mobility of the oxidized and intact proteins are not caused by charge effects since under the same conditions but in the presence of 4 M urea the proteins and their mixture have the same mobility (fig.2d–f).

3.2. *The PMR spectrum of the intact protein L11*

The PMR spectra of the intact protein and its derivatives are presented in fig.3.

The protein L11 contains 2 Tyr residues, in positions 7 and 61, and 4 Phe residues, in positions 37, 41, 66 and 68 [3]. Identification of the signals from the aromatic residues of the protein L11 has been done [13] and is represented in fig.3A. Proton signals of the 4 phenylalanine residues have a large range of chemical shifts from 6.0–7.6 ppm.

Moreover, in the spectrum of the protein L11 there are many signals in the region 0.7–0.1 ppm. These signals can be referred to methyl groups of aliphatic

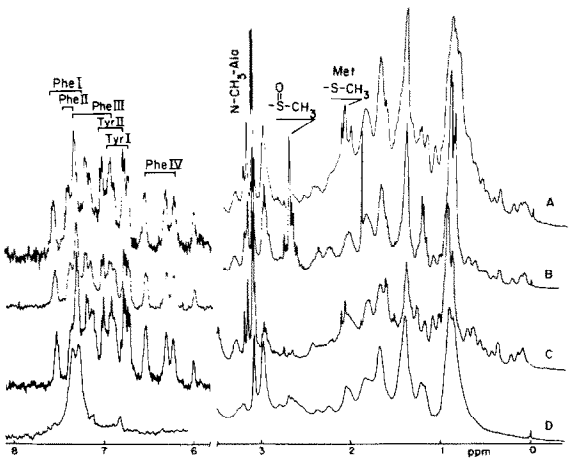

Fig.3. The PMR spectra of protein L11 and its fragments at 360 MHz. (A) Intact protein (1000 scans); (B) oxidized protein (5000 scans); (C) tryptic fragment in ^2H_2O, pH 6.6 (3000 scans); (D) fragment 38–141 (8000 scans). The aromatic regions are shown at an 8-fold increase in comparison with the high-field region. All spectra were recorded at 25°C.

amino acids whose side groups are in proximity to the planes of aromatic amino acid rings [6].

The wide range of chemical shifts of proton signals of aromatic residues and the presence of signals in the region 0.7–0.1 ppm are typical of the spectra of proteins having a compact tertiary structure [14].

3.3. *The PMR spectrum of the oxidized protein L11*

The PMR spectrum of the oxidized protein is practically the same as that of the intact protein. However, the signals of Val, Leu and Ile methyl groups (0.91 ppm) have a smaller width in the spectrum of the oxidized protein than in that of the intact protein.

Since all aromatic amino acid residues (Phe 37, 41, 66 and 68; Tyr 7 and 61) are located in the N-terminal part of the protein and the spectrum does not change in the region 6–7.6 ppm as a result of methionine oxidation, it can be assumed that the compact structure of the N-terminal part of the protein remains unchanged. At the same time the increase of intensity and decrease of the width of signal lines from Val, Leu and Ile residues indicate the increase of mobility of some of these residues. This suggests that upon oxidation, the structure of the C-terminal part becomes looser and the amino acid residues acquire greater mobility as it has been concluded from the electrophoretic data (see above).

3.4. *The PMR spectra of fragments 38–141 and 1–81*

Signals of methyl protons in the high-field part of the spectrum (0.7–0.1 ppm) are absent from the spectrum of the fragment 38–141 (fig.3). Proton signals of phenylalanine residues have close chemical shifts; this corresponds to their position in the spectrum of proteins in the unfolded state [14]. Thus, the fragment 38–141 constituting a considerable part of the protein sequence cannot form any noticeable tertiary structure by itself. At the same time, the spectrum of the fragment 1–81 (fig.3C) constituting a little more than half of the protein L11 sequence is similar to that of the intact or oxidized protein (fig.3A,B). A comparison of these spectra suggests that the N-terminal moiety of the molecule represents a well-formed compact structure.

When this paper was ready for publication a study on the renaturation of ribosomal protein L11 was published [15]. The spectrum of folded L11 (fig.3A [15]) coincides with that presented in fig.3 of our paper. Such a coincidence demonstrates that protein L11 in our hand has a well-formed tertiary structure though we have not used any special precautions for renaturation mentioned in [15]. Protein samples for the study were obtained by direct dialysis from the 5 M guanidine chloride solution against the buffer used.

4. Conclusions

The data presented above allow us to make the following conclusions:

(1) Protein L11 has a well-formed tertiary structure.
(2) The N-terminal moiety of the protein molecule constitutes the most compact and stable core in the protein L11 structure.
(3) The C-terminal part of protein L11 participates in the formation of the protein globular structure but unlike the N-terminal fragment cannot form a definite tertiary structure by itself.

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