STUDY OF THE SECONDARY AND TERTIARY STRUCTURE OF RIBOSOMAL PROTEIN S7 FROM *ESCHERICHIA COLI* IN SOLUTION

Z. V. GOGIA, S. Yu. VENYAMINOV, V. N. BUSHUEV* and A. S. SPIRIN

Institute of Protein Research, Academy of Sciences of the USSR, and **Institute of Biophysics, Academy of Sciences of the USSR, Poustchino, Moscow Region, USSR*

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1. Introduction

Ribosomal protein S7 is one of the 21 proteins of the small (30 S) subunit of *Escherichia coli* [1]. It consists of 177 amino acid residues [2]. It has been reported that the protein in the isolated state does not exhibit tertiary structure according to proton NMR spectra [3]. The large value for the radius of gyration obtained for protein S7 in solution (27 Å) agreed with the above data, indicating an elongated non-globular conformation [4]. However, a number of ribosomal proteins obtained in our laboratory turned out to possess a compact globular conformation in solution [5–7]. The small radius of gyration of protein S7, about 15 Å [5], directly indicated the presence of a compact globular structure. Preliminary scanning microcalorimetric experiments testified to the presence of a cooperative tertiary structure in this protein [8]. Here we report circular dichroism and nuclear magnetic resonance spectra of protein S7 in solution. It has been shown that this protein has a high content of secondary structure and a well developed tertiary structure.

2. Materials and methods

Protein S7 from the 30 S subunit of *E. coli* MRE-600 ribosomes was isolated and prepared as described for proteins S4 and S15 in our previous communications [6,7]. The identity, purity and homogeneity of protein S7 were checked by two-dimensional gel electrophoresis in urea, one-dimensional gel electrophoresis in sodium dodecyl sulfate, N-terminal group determination and amino acid analysis. The molecular weight (M_r) of the obtained preparation of protein S7 as determined by sedimentation equilibirum in standard solvent (0.1 M NaCl–0.03 M sodium phosphate, pH 7.0) was 19 000 ± 1000.

Absorption spectra were recorded with an EPS-3T Hitachi instrument. In order to estimate the extinction coefficient, the microtechnique of nitrogen determination [9] was used, assuming a nitrogen content of 18.8% [2].

Circular dichroism (CD) spectra were measured with a J41A JASCO instrument as described earlier for proteins S4 and S15 [6,7]. For calculation of the ellipticity the mean molecular weight of the residue (MRW) was assumed to be 111.5 [2].

Proton NMR spectra were recorded on a Bruker WH-360 Fourierspectrometer as described earlier for proteins S4 and S15 [6,7].

3. Results

3.1. *Extinction coefficient and secondary structure*

Fig.1 presents the ultraviolet absorption spectrum of protein S7 recorded in the buffer containing 0.05 M sodium phosphate, pH 7.0. It is seen that the spectrum corresponds to that of proteins with a high content of tryptophan residues. The absorption maximum is at 281.5 nm. The extinction coefficient ($A_{1\ cm}^{1\ mg/ml}$) is equal to 0.90 (±0.03). This extinction coefficient was used in all other experiments for the estimation of protein concentration.

The absorption spectrum and the extinction coefficient did not change upon addition of KCl, at least up to the concentration of 0.35 M.

Fig.2 presents the CD spectrum of protein S7. The shape of the curve and the ellipticity values in the far ('peptide') ultraviolet region indicate the presence of

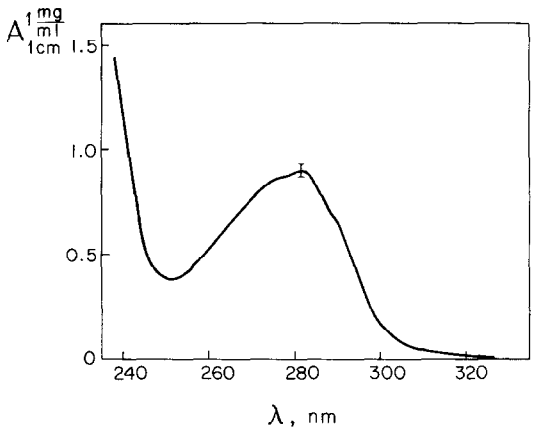

Fig.1. Absorption spectra in the range 235–330 nm of protein S7 in 0.05 M sodium phosphate buffer, pH 7.0.

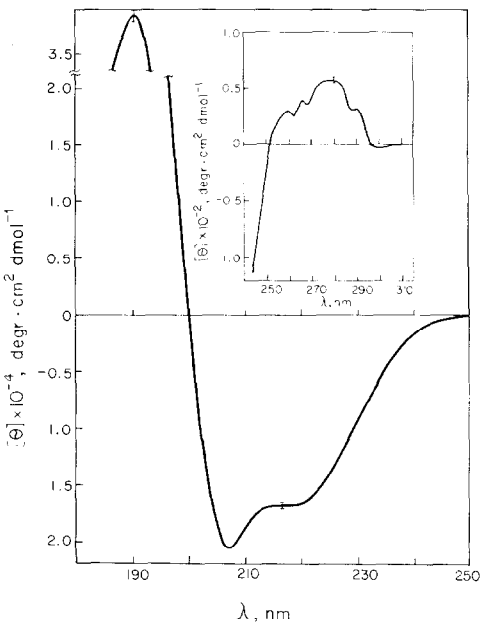

Fig.2. Circular dichroism spectra in the range 183–250 nm and 245–310 nm (insertion) of protein S7 in the 0.05 M sodium phosphate buffer, pH 7.0.

a well developed secondary structure in the protein studied. Calculation of the secondary structure content by the 'two-wavelength' method [10] using ellipticity values at 210 and 225 nm gave 53% α-helices and 64% β-form. Using the 'three-wavelength' method [11] and the ellipticity values at 210, 219 and 225 nm, 53% for the α-helix and 41% for the β-form were obtained. It is seen that the use of the reference spectra based on the CD spectra of globular proteins [10,11] gives full agreement between the values of the α-helical content calculated by the two methods and some discrepancy between the values for the β-form content in protein S7. In any case we can conclude from these data that the ribosomal protein S7

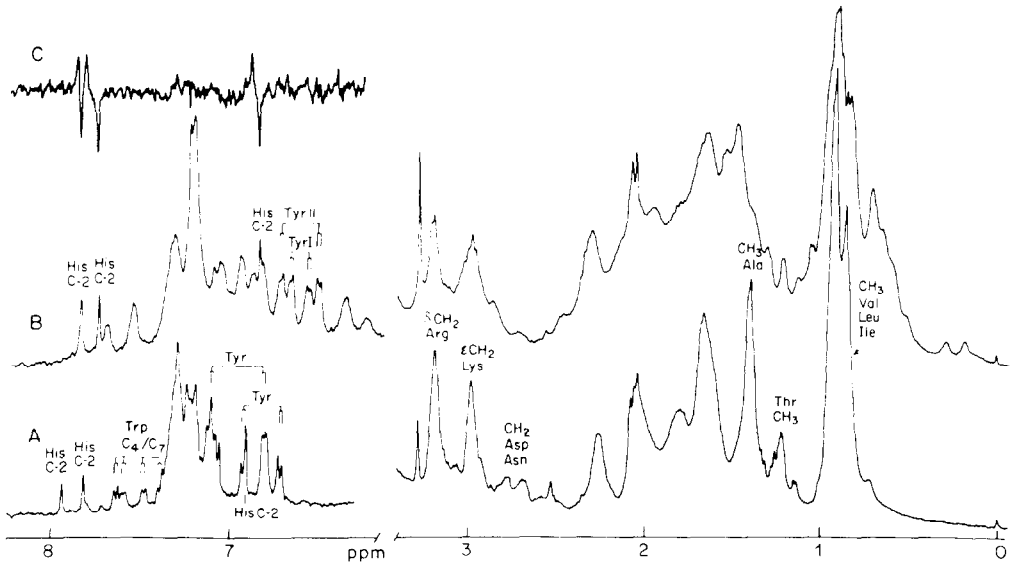

Fig.3. 360 MHz [^{1}H]NMR spectra of protein S7; 2 mg ml^{-1} in ^2H_2O containing 0.1 M NaCl–0.03 M sodium phosphate at 18°C: (A) in the presence of 2 M urea; (B) under the non-denaturing conditions, pD 6.7; (C) difference spectrum of protein S7 at pD 6.7 and 6.55 under non-denaturing conditions.

is characterized by a high content of secondary structure: about 50–55% α-helices and some considerable content of the β-form.

The presence of CD spectra with a fine, well-resolved structure in the near ultraviolet region is an argument in favour of the existence of a definite tertiary structure including chromophore groups of tryptophan, tyrosine and phenylalanine (fig.2).

3.2. *Tertiary structure*

Fig.3 shows the PMR spectra of protein S7 in the standard ^2H_2O buffer (B) and in the standard ^2H_2O buffer with 2 M urea (A).

The PMR spectrum of protein S7 under non-denaturing conditions (in buffer B) exhibits a large number of signals from methyl protons in the extreme high field (in the range 0.8–0.15 ppm). These signals can be attributed to methyl group protons of apolar aliphatic residues whose side chains are in close proximity to the planes of aromatic residue rings in the protein S7 molecule [12]. Therefore the presence in the protein S7 PMR spectrum of a large number of methyl proton signals in the extreme high field indicates the spatial proximity of many apolar aliphatic residues to aromatic residues; this testifies to a compact shape of the folded protein S7 structure in solution.

Signals in the low field (from 6.0 to 8.0 ppm) pertain to protons of aromatic amino acid residues: four tyrosines, five phenylalanines, two tryptophans and three histidines of protein S7. Taking into account a characteristic shift of histidine signals upon pD change, singlet signals (1 proton each) at 7.84, 7.44 and 6.84 ppm were attributed to C-2 protons of the three histidine residues. The signal at 6.84 ppm can be attributed to His 151, since an extreme high-field position of the C-2 histidine signal can be explained by the effect of ring currents of the near aromatic residues Phe 149, Tyr 152 and Trp 154.

The use of double resonance allowed us to show that doublet signals at 6.54 and 6.65 ppm as well as signals at 6.70 and 6.45 ppm pertain to two tyrosine residues, respectively. Other signals in the aromatic region were not attributed owing to strong overlapping of the lines. This requires further study.

The wide range of chemical shifts of aromatic residues and histidine resonance lines in the protein S7 spectrum testifies of a specific stable environment of these residues. This suggests a compact globular structure of protein S7 under these conditions.

In the presence of 2 M urea (fig.3A) the protein spectrum has narrower and better resolved resonance lines of aliphatic and aromatic residues. Signals in the extreme high field characteristic of the compact globular structure are absent. This indicates that at 2 M urea the globular state disappears and protein unfolding takes place.

4. Discussion

The results presented testify that the isolated protein S7 in solution obtained in our laboratory has a globular conformation characterized by a high content of secondary structure (at least about 50% α-helices) and a well developed tertiary structure. This conclusion agrees well with our previous data on a high compactness of protein S7 (R_g = 14.5 Å) [5] and on the presence of a cooperative transition at melting [8].

A relatively low stability of the compact tertiary structure of the isolated protein S7 should be noted. Thus, in the presence of 2 M urea it is already practically disrupted (fig.3A). Microcalorimetric data indicate a rather low melting temperature of this protein in solution, near 40–45°C [8].

A CD spectrum of protein S7 has been earlier reported by other authors [13]. It differs considerably from ours in the range 207–230 nm. In our opinion, the main reason lies in the calibration procedure used for CD instruments: the authors [13] noted that the CD spectra of standard proteins (myoglobin, lysozyme and ribonuclease) obtained with their instrument differed from those published earlier [11], while the CD spectra of the same proteins recorded with our instrument completely coincide with those published earlier.

As to the published data on the absence of indications for tertiary structure in the PMR spectrum of protein S7 [3] as well as on its elongated conformation in solution according to small-angle X-ray scattering [4], we believe that these discrepancies are due to the denatured state of the protein preparations used by those authors.

In this connection we would like to mention a recent communication of Kime et al. [14] on renaturation of ribosomal protein L11 followed by means of NMR. The authors indicate that the urea-denatured preparations can serve to obtain two different protein forms in non-denaturing buffers – an unfolded one and the other one folded, depending on the renatura-

tion conditions. We are glad to corroborate the authors' observations that the preparation of folded (i.e. well renatured) forms of ribosomal proteins is promoted by a low protein concentration in the process of renaturation and by the presence of salt in the buffer used for protein solutions.

We would also like to emphasize the complete correlation observed by us between the tertiary folding according to PMR data and the small values of radii of gyration obtained for the same preparations of ribosomal proteins. We think that small values of radii of gyration will always mean a high degree of tertiary folding as has already been shown for proteins S15 [6], S4 [7], S7 (this communication and [5]), S8 [5,15] and S16 [5,15,16].

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