GLOBULAR STRUCTURE OF THE SMALL PROTEINS L29 and L30 FROM *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

At present the globular structure of several proteins (S4, S7, S8, S15, S16 [1]) from the small subunit has been verified. A detailed study of some proteins from the large subunit, L7 [2], L11 [3,4] and L25 [5], also indicates a compact tertiary structure of these proteins.

Among the 33 proteins of the large subunit, two small proteins L29 (63 amino acids, M_r 7272 [6]) and L30 (58 amino acids, M_r 6411 [7]), as will be shown below, have a compact globular structure. These small proteins renature easily; their tertiary structure folds and is maintained for their monomers without additional covalent (e.g., S–S bridges) bonds.

2. Materials and methods

Hydrodynamic studies were carried out in a Beckman E (USA) ultracentrifuge with a scanning optics system. CD spectra were recorded on a J-41A (JASCO, Japan) instrument. Proton magnetic resonance (PMR) spectra were recorded on a WH-360 (Bruker, FRG) spectrometer in a pulse mode with a following Fourier transform in 5 mm tubes. The concentration was in a 1–2 mg/ml range.

Lyophilized proteins were dissolved with guanidyl–HCl and dialyzed against chosen buffers at +4°C immediately before the experiment.

3. Results and discussion

3.1. *Velocity sedimentation*

We found the sedimentation and diffusion coefficients after extrapolation to zero concentration to be $s^0_{20} = 1.0$ S, $D^0_{20} = 15.6 \times 10^{-7}$ m^2/s for the L30 protein and $s^0_{20} = 1.17$ S and $D^0_{20} = 15.30 \times 10^{-7}$ m^2/s for the L29 protein. The M_r-value from the sedimentation and diffusion coefficients is 6300 for L30 and 6900 for L29, i.e., there is a good correspondence to the values calculated from their amino acid composition.

The frictional ratio, calculated from the s and D values is $f/f_o = 1.10$ and practically coincides with that for classicial globular proteins (such as myoglobin). Hence, it can be concluded from the above data that L29 and L30 in solution are monomeric globular proteins.

3.2. *CD measurements*

CD spectra indicate convincingly that the L29 and L30 proteins have a high content of secondary structure (fig.1). The $[\Theta]$ value is especially high for the L29 protein, which is a very helical (72% helices) protein. The crossovers of the zero line at 200 nm for L29 and 201.3 nm for L30 and the high ratio of $[\Theta]_{max}/[\Theta]_{min}$ [8] are also in line with a highly ordered secondary structure. There is only one Phe residue in the L29 and L30 proteins [5,6]. A well pronounced spectrum in the near UV-region for the L30 protein with positive maxima at 261 and 267 nm (fig.1, insert) indicates a highly asymmetrical environment of the Phe residue. Such an asymmetry can exist only in a compact tertiary structure where the Phe residue is buried. Taking into consideration the high content of secondary structure, the asymmetrical environment of the single phenylalanine residue and the small amount of amino acids in the protein, it may be concluded that L30 has a globular structure.

The CD spectrum in the near UV-region of L29 indicates a less asymmetrical environment of the Phe

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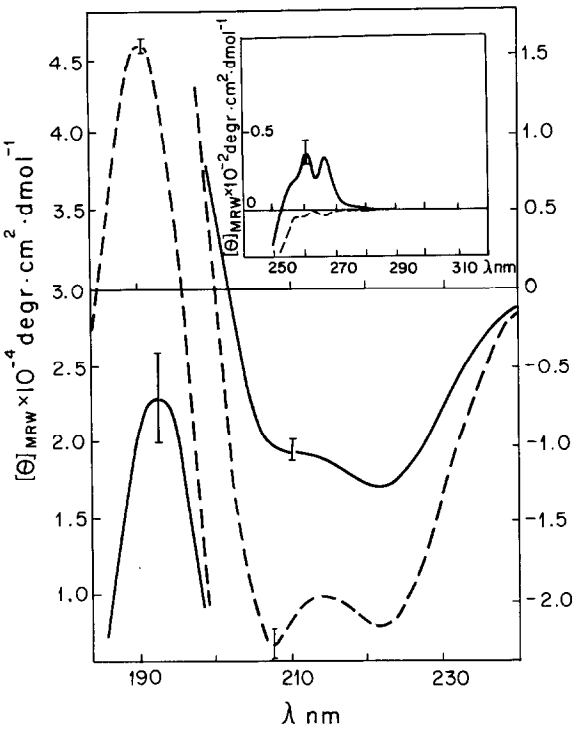

Fig.1. CD spectra of L30 (——) and L29 (– – –) proteins in 0.1 M Na-phosphate buffer (pH 7.0). Positive [Θ] scale at the left. The near UV-spectra are shown in the insert.

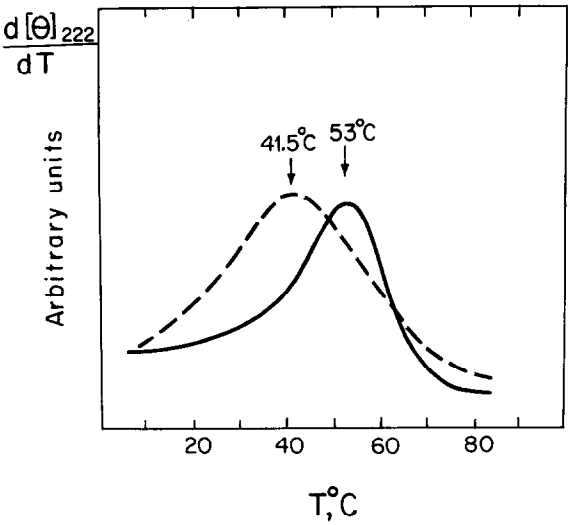

Fig.2. Melting curves of L29 (– – –) and L39 (——) proteins. Results are given in a differentiated form of the melting curves at 222 nm.

residue and as a consequence it may probably be exposed. The melting curves (fig.2) evidence that the L30 protein is more stable and has a more cooperative structure as the melting temperature is higher and the width of transition is narrower for L30 than the same parameter for the L29 protein.

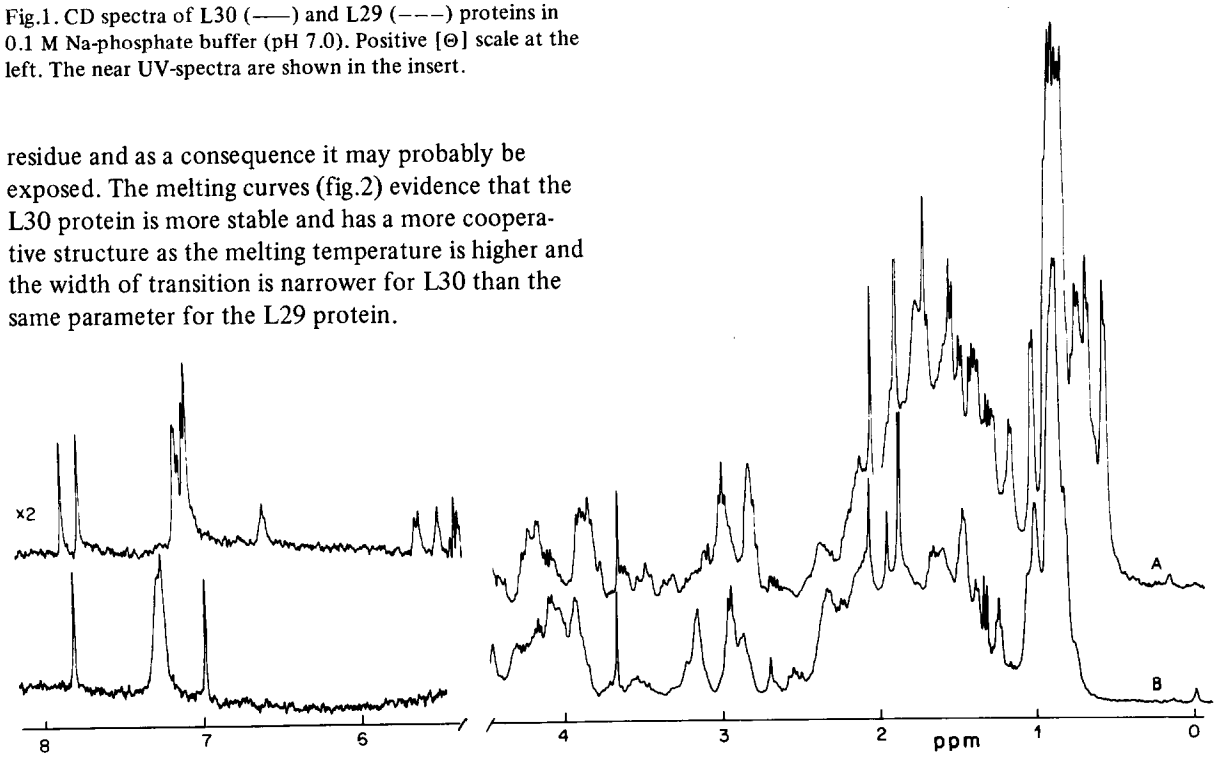

Fig.3. 360 MHz PMR spectra of L29 (B) and L30 (A) in the 50 mM Na-phosphate buffer (pH 6.7), 0.3 M potassium chloride buffer in D_2O.

3.3. *PMR spectra*

More direct information on tertiary structure can be obtained from PMR data. The PMR spectra of proteins in the denaturated and folded states can be well distinguished. The appearance of new signals for folded proteins characterize the environment of amino acids while the signal width (in particular) reflects the immobility of amino acid side groups [9].

The PMR spectra of the L30 and L29 proteins are shown in fig.3. A number of signals are shifted to an unusually high field (0.8–0.5 ppm) and indicate a compact, folded structure of the L30 protein. These signals, as a rule, pertain to protons of the methyl groups of aliphatic amino acids, and their interreaction in the ring current of the aromatic residues causes this shift [9]. The integral intensity of these resonances in the L30 spectrum corresponds to >36 protons. We believed therefore that ~6 of the 15 apolar amino acids are very close to the single Phe residue. This can indicate the rigid fixation of the phenylalanine. This conclusion is corroborated by the perturbation in the aromatic region of the spectrum (fig.3A). Signals in the 5–5.8 ppm region confirm the compact structure as in the unfolded state there are no such resonances. It is of special interest that in the spectrum of the L30 protein there are practically no resonances at 3.2 ppm, where there should be signals from the δ-CH_2 group of arginines due to the fact that the protein has 6 arginines. In the protein denaturated by urea (not shown) there is an intensive signal at 3.2 ppm. It seems that most of the Arg residues in the protein L30 are immobilized.

The spectrum of L29 has no such well-defined features as the spectrum of L30. The analysis of the L29 spectrum permits to conclude that the only Phe residue is to some extent exposed. The number of resonances in the high field is much less, the perturbation in the aromatic region is also less. At the same time more broad signals in the spectrum of the undenaturated protein L29 indicate a structural compactness of this protein.

Together, these data indicate a compact globular structure of the L29 and L30 proteins.

An easy renaturation, a monomeric state in solution and the low M_r of these proteins can be of interest for the study of protein structure folding.

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