A PHYSICAL STUDY OF ACIDIC RIBOSOMAL PROTEINS FROM *ARTEMIA SALINA*, *BACILLUS SUBTILIS* AND *MICROCOCCUS LYSODEIKTICUS*

A. T. GUDKOV, S. Yu. VENYAMINOV, R. AMONS+, W. MÖLLER+ and T. ITOH*

Institute of Protein Research, Poustchino, Moscow Region, USSR, +Department of Medical Biochemistry, Sylvius Laboratories, State University of Leiden, The Netherlands and **Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima, Japan*

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1. Introduction

Ribosomal L7/L12-type protein has been the subject of many investigations. The acidic protein is present in multiple copies in ribosomes of both prokaryotic and eukaryotic organisms, and is intimately involved in elongation factor dependent processes of protein biosynthesis (reviews [1,2]).

Comparative studies were primarily focused on the amino acid sequence of various L7/L12-type proteins [3,4]. *Escherichia coli* L7/L12 protein occurs in aqueous solutions as a dimer with an elongated shape [5–7]. It possesses a large amount of α-helices [5,8,9]. Preliminary ultracentrifuge studies [10] and a secondary structure prediction [11] of eukaryotic eL12 protein indicate properties similar to L7/L12.

Here we present some hydrodynamic and circular dichroism studies on *A. salina* eL12 protein, *B. subtilis* BL9 and *M. lysodeikticus* MA1 proteins. These studies show that all proteins in solution are dimers, have high content of the secondary structure and high thermostability. At the same time there are differences in their properties: eukaryotic proteins (eL12 from *A. salina*) do not bind to the L10 from *E. coli*. EL12 protein has a more symmetrical shape than other types of acidic ribosomal proteins from prokaryotes.

2. Materials and methods

In the study we used the following buffer solutions: (A) 10 mM Tris–HCl (pH 7.5), x M KCl, x = 0.1, 0.3, 0.6, 1.0, 2.0; (B) 10 mM Na-phosphate (pH 7.3).

The concentration was determined from the nitrogen content as in [12]. *A. salina* eL12 protein was isolated from cysts as in [10]; BL9 and MA1 proteins were isolated as in [13]. Sedimentation velocity was studied in the Beckman E (USA) and UCA-QM (USSR) ultracentrifuges. Equilibrium sedimentation experiments were carried out with a MOM centrifuge (Hungary) by the method of Yphantis [14]. Circular dichroism measurements were done with a J-41A instrument (Japan).

3. Results and discussion

Sedimentation velocity was studied in buffer A with 0.3 M KCl. For *A. salina* eL12 protein we obtained $s_{20,w} = 1.85 \pm 0.05$; $D_{20,w} = 7.5 \pm 0.2$; $M_{s,D} = 24\,000 \pm 1000$. For *E. coli* L7/L12 $s_{20,w} = 1.60 \pm 0.05$; $D_{20,w} = 5.5 \pm 0.5$; $M_{s,D} = 24\,000 \pm 1000$ [7].

Sedimentation and diffusion coefficients for protein eL12 are higher than those for protein L7/L12 with the same M_r-value. Hence it follows that the eL12 dimer in solution has a more symmetric shape than the L7/L12.

The M_r of the protein eL12 dimer and conditions of its existence in solutions were studied in more detail by equilibrium sedimentation. The M_r (mean value 23 000 ± 1000) corresponds to the dimer form of eL12 and does not depend on the ionic strength and pH in the range 0.1–2 M KCl (pH 7.5–10.0). In the presence of 1 M ethanol the M_r changes.

Since protein eL12 is a functional analog of L7/L12 protein, we attempted to test the complex formation of eL12 with *E. coli* L10 protein. Except

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for a weak tendency to interaction in equilibrium experiments, no complex formation was observed. Neither does the complex form at gel filtration of the mixture of eL12 and L10 on Sephadex G-100, though under analogous conditions L7/L12 and L10 give a 4:1 complex [15].

According to equilibrium sedimentation, proteins BL9 and MA1 are dimers in solution (their M_r = 21 000–23 000). These proteins were mixed with L10 and the M_r was studied by equilibrium sedimentation. The results (M_r = 46 000–48 000) show that BL9/L10 and MA1/L10 complexes are formed. Due to the small quantity of BL9 and MA1 we could not isolate the complex from the mixture and determine the exact stoichiometry.

3.1. *Circular dichroism measurements*

The extinction coefficient $A_{1\,cm}^{mg/ml}$ at 227 nm is 0.38 ± 0.01 in buffer B for *A. salina* eL12 protein. It is seen from circular dichroism spectrum of protein eL12 (fig.1) that the protein has a high content of secondary structure and, in particular, a high degree of helicity.

In the CD spectrum in the near ultraviolet region (fig.1, insertion) there are maxima pertaining to the Phe residues (261 and 267.5 nm) and a maximum pertaining to the Tyr residue (281 nm). The presence of these maxima points to the asymmetric environment of these residues and, therefore, to a definite tertiary structure of protein eL12 in solution. There are qualitative differences in the CD spectra of proteins eL12 and L7/L12. The ratios of positive and negative extremes differ; in the eL12 spectrum the crossover (fig.1) is shifted towards the short wavelengths. These differences can indicate the quality and/or length of α-helical fragments. It can be assumed that in protein L7/L12 the α-helices are less distorted or larger.

A heat denaturation study of protein eL12 revealed a remarkably high thermostability. The ellipticity value of λ = 222 nm decreases only by 30% at heating to 93°C (fig.2) and is practically completely restored after cooling, i.e., the process is reversible. We did not succeed in reaching the denaturation temperature (not shown) in calorimetric studies either (the protein solution was heated to 112°C). CD measurements have shown that protein eL12 has, after cooling in the calorimeter, an initial secondary structure.

Since there are indications on the increase of protein thermostability at binding of 2-valent cations (e.g., Ca-binding proteins), we studied protein thermostability upon addition of EDTA, but did not

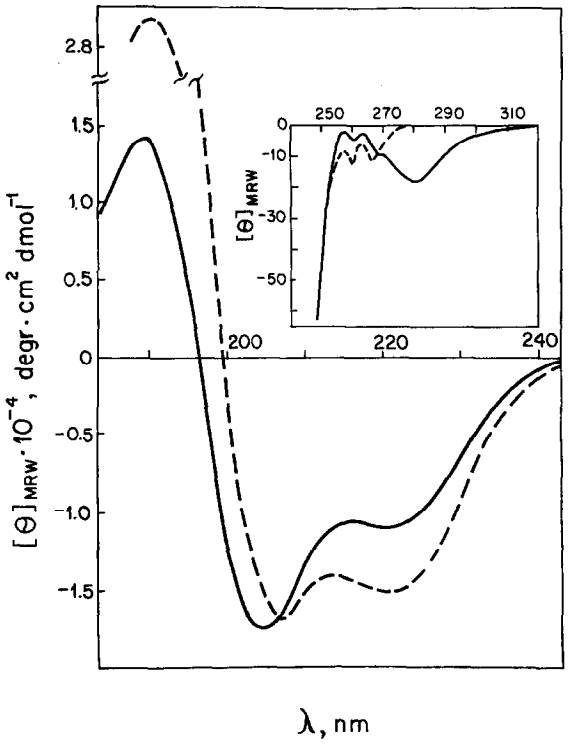

Fig.1. (——) CD spectrum of eL12 in buffer B; (– – –) CD spectrum of L7/L12. The insertion represents the SD spectra of eL12 and L7/L12 proteins in the near UV-region. The spectra are averaged from several measurements.

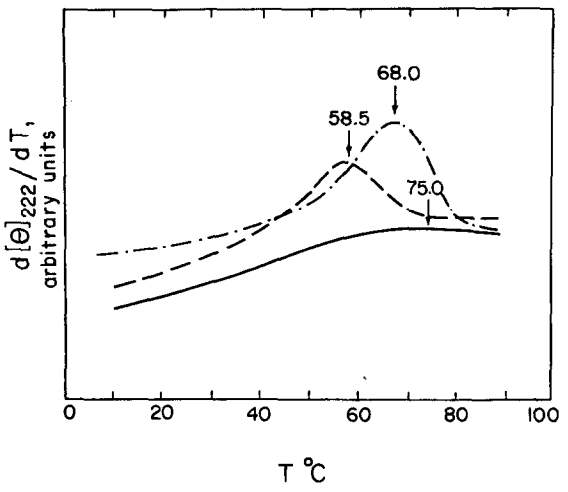

Fig.2. Melting curves of proteins BL9 (– – –), MA1 (–·–·–) and eL12 (——).

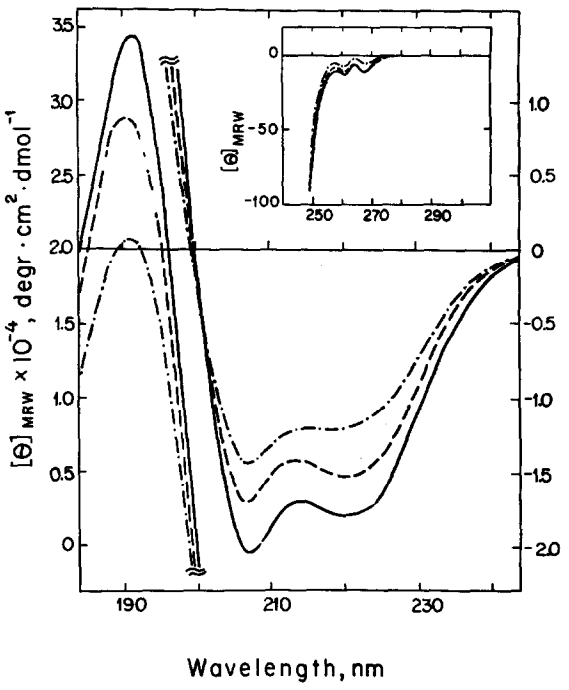

Fig.3. CD spectra of proteins MA1 (–·–·–), BL9 (——) and L7/L12 (– – –) in 0.1 M sodium phosphate buffer (pH 7.0). [θ] values for wavelengths of 190–200 nm are given on the left and those for 200–250 nm on the right.

observe any changes in the presence of 5 mM EDTA.

CD spectra in the far and near ultraviolet region (fig.3) of BL9 and MA1 are qualitatively similar to the spectrum of L7/L12 from *E. coli* ribosomes. Temperature denaturation studied by the CD method (fig.2) indicates a thermostability similar to L7/L12 proteins [9].

L7, BL9 and MA1 proteins have a high degree of homology (fig.4), similar physical properties and form a complex in solution with L10 from *E. coli* ribosomes.

The acidic protein B-L13 from *Bacillus stearothermophilus* [16] forms a dimer in solution and a complex with B-L8 protein, a probable analog of L10 protein from *E. coli*. This complex can restore the full activity of 50 S core particles from *E. coli* ribosomes [16].

Analysis of primary structure of proteins eL12 and L7 reveals little overall homology, although a few partial sequences in L7 may be retained albeit in other locations of the eL12 molecule. The significance of this correspondence is not clear but could be due to a reshuffling of exons during the course of evolution [17]. Alternatively the presence of Glu- and Ala-rich sections in both eL12 and eL7 may reflect common structural features.

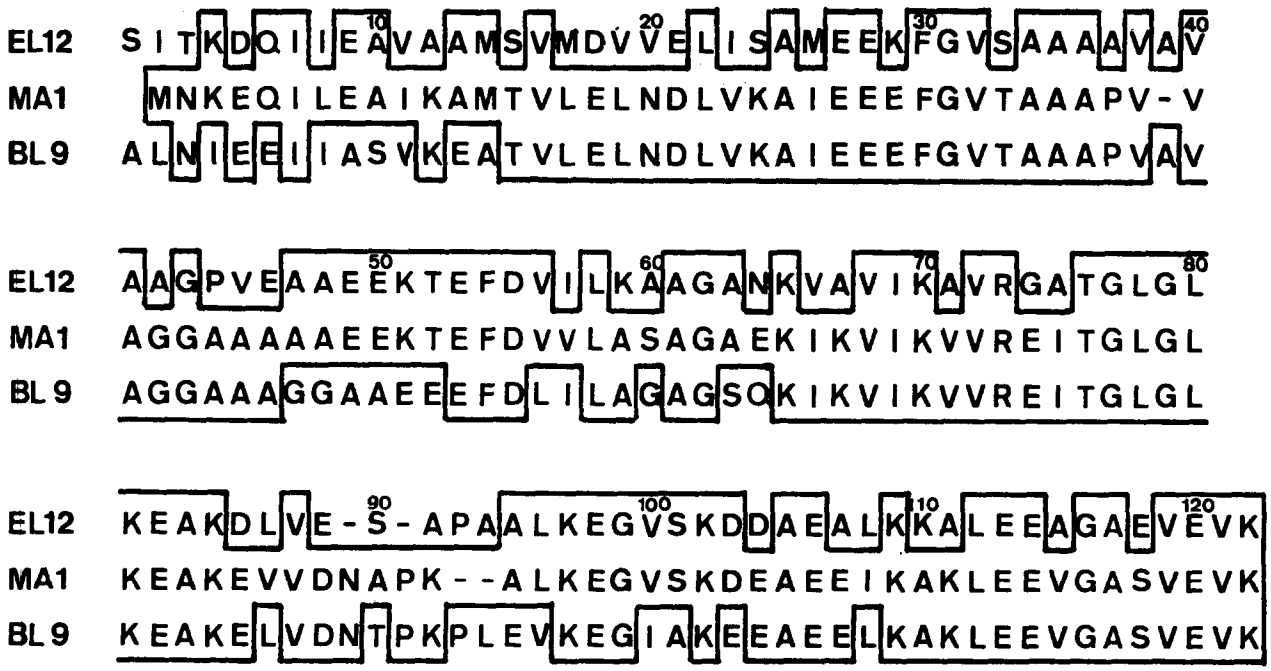

Fig.4. Primary structures of proteins L7, BL9 and MA1. Identical regions are boxed.

It is remarkable that throughout evolution, the large subunit of ribosomes contains at least one dimer of an acidic protein of M_r = 22 000–24 000, high secondary structure and thermal stability. Since in procaryotes this acidic protein maps as a stalk extending from the large subunit [18], it would be interesting to see whether eL12 also maps in the stalk region observed for the 60 S subunit of *Artemia salina* [19].

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