CHARACTERIZATION OF AN ACIDIC PROTEIN IN 50 S RIBOSOMES OF *E. COLI*

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1. Introduction

The presence of an alanine rich, acidic protein, called ribosomal A-protein, has been reported in 70 S ribosomes of *E. coli* [1, 2]. In this communication, evidence is presented that this protein is an integral part of the 50 S ribosome and is estimated to contain *two* acidic peptide chains of molecular weight 19,000. The possibility that A-protein is involved in peptide bond formation [3] is discussed.

2. Materials and methods

70 S Ribosomes from *E. coli*, strain MRE 600, were prepared as described by Gesteland [4], except that the cells were broken by glassbeads; the standard buffer consisted of 10 mM $MgAc_2$, 5 mM tris (Cl), pH 7.4 and 6 mM mercaptoethanol. Prior to extraction of the protein, 70 S ribosomes were washed with 0.5 M NH_4Cl solutions as described earlier [1].

Dissociation of 70 S ribosomes into 50 S and 30 S ribosomes was carried out by dialysis overnight against a solution of 0.1 mM $MgAc_2$, 5 mM tris (Cl), pH 7.6 and 6 mM mercaptoethanol. A 20 ml solution containing 300–500 mg ribosomal subparticles was layered on a linear sucrose gradient (10–25% (w/v)) in the same buffer and centrifuged for 4 hr at 40,000 rpm at 3° in a Spinco B IV zonal rotor. The two types of subparticles were concentrated in a Diaflo apparatus, filter PM-30, and subsequently dialyzed overnight in the cold against 10 mM $MgAc_2$, 5 mM tris (Cl), pH 7.4, 6 mM mercaptoethanol. The subparticles were pelleted in a Spinco Ti-50 rotor at 50,000 rpm for 5 hr and stored at −25°. Prior to extraction of the 50 S proteins, 50 S ribosomes were washed once with 0.5 M NH_4Cl solution [1].

Ribosomal proteins were extracted by the method of Waller and Harris [5]; they were recovered from the supernatant containing 66% acetic acid by passage through Sephadex G25, equilibrated with 1% of formic acid. The proteins were lyophilized, dissolved in water, lyophilized and stored at −25°.

50 S- and 30 S acidic proteins were isolated from total 50 S- and 30 S proteins according to the procedure used to isolate 70 S acidic proteins from total 70 S proteins by CM-cellulose chromatography [1, 2].

The 70 S acidic proteins could be further subdivided by chromatography on DEAE Sephadex, A 50, and gradient elution with 0.2–0.5 M KH_2PO_4–K_2HPO_4, 6 M urea, pH 5.7 buffer rather then by DEAE cellulose chromatography [1]. A-protein elutes first, then 30 S-1 protein, followed by 30 S-2 protein [6]. The latter was identified on the basis of comparative amino acid composition, molecular weight, number of copies and band position on the gel [6].

Molecular weights were determined with a Spinco, model E ultracentrifuge, equipped with interference optics. The high-speed, short column method of Yphantis [7] was used. The partial specific volume of A-protein was taken as 0.751; the figure was calculated from the amino acid composition and the specific volume of the constituent amino acids [8].

The dichroic absorption spectrum of the protein was measured with a Roussel-Jouan dichrograph, model CD-185, in cells of 0.5 mm and 1 mm path length. Circular dichroism results are reported in terms of $[\theta]$, the mean residue ellipticity in units of deg.cm^2/dmole. The mean residue weight of A-protein was taken as 117. The protein concentration used was 0.180 g per liter in 0.01 M Na_2HPO_4 buffer, pH 7.5.

The concentration of the protein was estimated by

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the microbiuret method [9] using crystalline ox insulin (Allen and Hanburys Ltd) as a standard. The concentration of the insulin standard was estimated using an $A_{1\,cm}^{1\,mg} = 1.03$ at 275 nm (0.01 N HCl). As a check on the validity of the concentration determination, an amino acid analysis assay was run on a weighed aliquot of lyophilized A-protein. The calibration mixture of amino acids, 1 μmole each, was also subjected to conditions of acid hydrolysis. The results of the amino acid assay gave 96% of the microbiuret value for the content of A-protein. The mass ratio of A-protein to total 50 S or 70 S protein was estimated by means of the microbiuret reaction which gave the total amount of protein in the lyophilized samples recovered from the column. Losses due to the recovering procedure were neglected but should not seriously affect our conclusions about the number of copies per ribosome.

Amino acid analyses were run with an automatic Amino acid Analyzer, Beckmann, Spinco, 120 C. Samples were hydrolyzed with 6 N HCl for 22 hr at 110° in evacuated tubes. Performic acid oxidation was performed for 3 hr at room temperature.

Dimerization of A-protein was observed after a solution of 0.06% of the protein in 0.01 M tris (Cl), pH 7.6, 0.01 M $MgAc_2$, 0.15 N KCl was saturated with oxygen and left at 5° for 3 hr. Gel electrophoresis was performed in 15% polyacrylamide gels at pH 3.5, 8 M urea [10].

3. Results

Plate I gives the pattern of a gel, loaded with 50 S acidic protein referred to as A-protein (50 S). The protein moves as the two bands of A-protein [1], while no bands of 50 S acidic protein overlap with those of 30 S acidic protein.

The amino acid compositions of table 1 confirm that A-protein is the only 50 S acidic protein. With exception of a difference in the content of arginine, the agreement between the two analyses is reasonable

Table 1
Amino acid composition of acidic proteins.
Number of moles per 100 moles amino acid recovered

Amino acid	A-protein*	A-protein (50 S)	30 S-2-protein (this paper)	30 S-2-protein (Craven et al. [6])
Asp	7.1	6.9	11.4	12.6
Thre	3.4	3.8	4.8	5.3
Ser	4.2	4.4	3.5	3.1
Glu	13.1	13.1	14.0	15.8
Pro	2.3	2.2	4.6	3.9
Gly	6.9	6.6	3.8	4.0
Ala	19.8	19.8	13.0	11.0
Val	12.3	11.9	7.0	6.6
Ileu	4.8	4.7	4.0	4.5
Leu	6.8	7.8	3.9	4.0
Tyr	1.0	0.8	1.8	2.8
Phe	2.4	3.0	2.6	3.2
Lys	9.2	9.2	6.7	4.3
His	0.8	0.8	4.3	4.5
Arg	1.8	2.7	11.3	10.6
Trp	0.0	0.0	–	–
Meth	2.9	2.3	3.1	3.1

* Taken from [1].

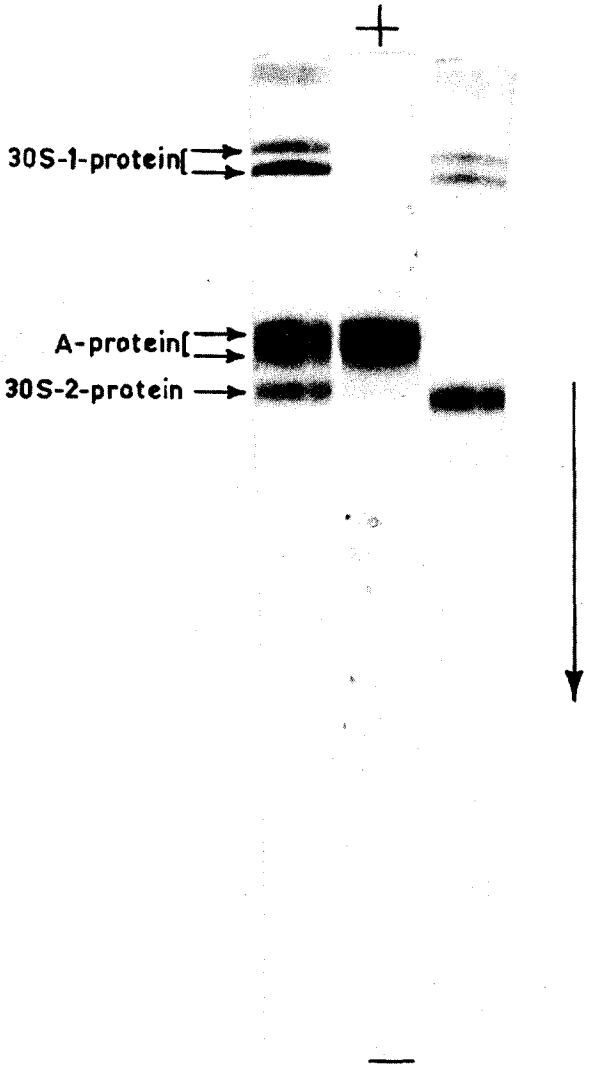

Plate 1. Polyacrylamide gelelectrophoresis of acidic ribosomal proteins. Cathode at bottom. Migration from top to bottom.
1) 70 S acidic protein
2) 50 S acidic protein
3) 30 S acidic protein; the two top bands vary in relative and absolute intensity depending on the procedure used to wash the 30 S particles; the extracted protein resembles 30 S-1 [6] but is not identical; lowest band, 30-S_2 protein.

when compared for instance to the composition of the acidic protein, 30 S-2, that moves slightly ahead of A-protein.

The amount of this protein has been estimated as approximately 5 g per 100 g of total 70 S protein [1]. Using a molecular weight of 19,000 for the protein, of 1.65×10^6 for 16 S + 23 S RNA [11], and a 1 to 2 weight ratio for protein to RNA in 70 S ribosomes washed with 0.5 M NH_4Cl, 2.2 copies of A-protein per 70 S ribosome is calculated. Similarly, starting from 0.5 M NH_4Cl washed 50 S ribosomes, a value of 2.0 ± 0.4 molecules of A-protein per 50 S ribosome is obtained by chromatographic analysis of 50 S proteins. For comparison, the number of copies of the acidic 30 S-2 protein was found to be 1.0 ± 2, somewhat higher than the reported value of 0.75 [12].

The molecular weight of A-protein in neutral aqueous solutions was in the range of 19-26,000 ($\bar{M}_w$ = 23,000), the lowest value being approached towards the meniscus of the centrifuge cell (table 2). To test for possible SH-dependent interactions, a sample of A-protein (50 S) was oxidized with performic acid. As a result the protein gave a molecular

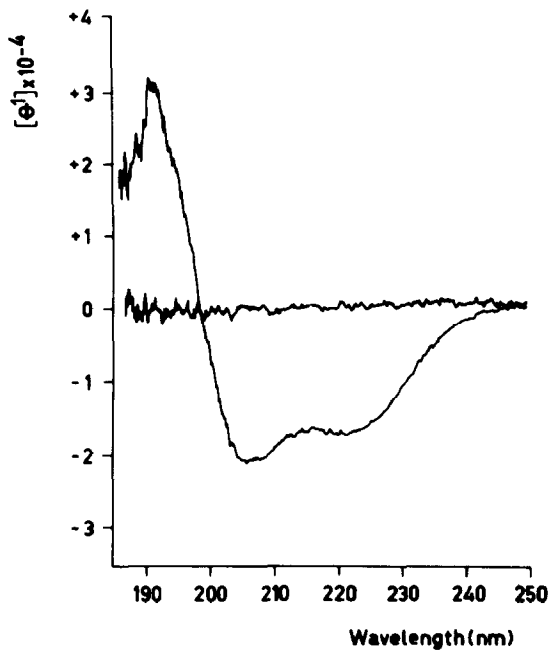

Fig. 1. Circular dichroism spectrum of A-protein.

Table 2
Molecular weights measured under various conditions.

Protein	Molecular weight	Solvent	Treatment
A-protein (50 S)	23,000 ± 1000	1	
A-protein (50 S)	19,000 ± 1000	2	Performic acid oxidized
A-protein (50 S)	39,000 ± 1000	2	Air (O_2) oxidized
A-protein (70 S)	21,000 ± 1000	1	
30 S-2-acidic protein, code Craven et al. [6]	18,000 ± 1000	1	

Concentration protein 0.6 mg/ml; length of liquid column 2.5 mm; speed 44,770 rpm.
Solvent 1: 0.01 M tris (Cl), pH 7.6, 0.01 M $MgCl_2$, 0.15 N KCl, 0.006 M β-mercaptoethanol.
Solvent 2: 0.01 M tris (Cl), pH 7.6, 0.01 M $MgCl_2$, 0.15 N KCl.

weight of 19,000 uniformly throughout the centrifuge cell. When a sample of A-protein (50 S) was oxygenated under mild conditions which favor the formation of -S-S-bridges, the monomeric form was presumably converted to a dimer of molecular weight of 39,000. The results of the various molecular weight determinations are given in table 2.

Tryptic peptide analysis of A-protein yielded about twenty peptides, *one* of which contained cysteine, A 14. Its molar composition was 1 lysine, 1 alanine, 2 leucine, 2 glycine, 1 threonine, 1 cysteine.

The combined results together with our recent peptide studies of the protein support the assumption that in free solution a monomer-dimer equilibrium can exist between the two chains of closely related primary structure. Actually the isoelectric points of the material corresponding to each band differ by about 0.2 of a pH unit (pH 4.7 and 4.85 as determined by isoelectric focussing).

From the dichroic absorption at 208 nm and using method 1 of Greenfield and Fasman [13], a value of 60 ± 5% α-helix has been derived for A-protein (fig. 1). The value of $\theta 208/\theta 222$ was 1.10, that of $\theta 217/\theta 222$ was 0.97 corresponding to about 20% β-structure. A similar helix estimate has been obtained earlier by Dzionara [14]. Basic ribosomal proteins are reported to have much lower contents of α-helix, namely some 25% [15, 16].

4. Discussion

The occurrence in 50 S ribosomes of *two* similar acidic peptide chains is remarkable, particularly because so far none of the basic ribosomal proteins seem to occur with a frequency exceeding *one* copy per ribosomal subparticle [12]. This acidic protein cannot be released from the ribosomes by washing the subparticles with solutions of 0.5 M NH_4Cl [1] or $(NH_4)_2SO_4$ [6]. These are conditions used to release supernatant enzymes such as ribonuclease 1 [17], or T- and G elongation factors [18]. In this respect A-protein behaves as an integrated 50 S ribosomal enzyme. It is interesting that peptidyl-transferase activity is ribosome-associated [3]. Possibly the two chains of A-protein may form the two enzymic sites, necessary for aligning and binding the *two*-CpCpA-termini of peptidyl-tRNA and aminoacyl-tRNA during peptidyl transfer [19]. One indication for an enzymic function of 50 S acidic protein, is the finding of Traub and Nomura [20] that for Poly U-induced polyphenylalanine synthesis, an acidic 50 S protein fraction needs to be present in the artifical reconstituted particle; however 50 S particles which lack this acidic fraction are able to partially bind tRNA non-specifically. This finding has been extended by Stöffler [21], who found that in contrast to other ribosomal proteins, antibodies made against A-protein strongly inhibit Poly U-induced polyphenylalanine synthesis, whereas little effect is observed on the ribosome-tRNA binding reaction.

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