THE ORIGIN OF HIGH MOLECULAR WEIGHT PROTEINS IN RIBOSOMAL PREPARATIONS OF *BACILLUS LICHENIFORMIS*.

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1. Introduction

E. coli ribosomes and free 30S subunits contain a number of acidic and relatively large proteins, which are lost during washing with a buffer containing 0.5 M or 1.0 M ammonium salt and therefore are generally regarded as adhering supernatant proteins [1–3]. On the other hand, it was suggested by Subramanian in a recent paper [4] that these high molecular weight proteins should not be considered as supernatant proteins but rather as ribosomal constituents, since they could not be washed off with a low-salt buffer and subsequent centrifugation through a sucrose cushion. Moreover, only a few of them could be detected in an electropherogram of the supernatant protein fraction [4].

Since in *B. licheniformis* the majority of the ribosomal material is associated with the membranes [5], this organism provides a good system to test the hypothesis that high molecular weight proteins in ribosomal preparations are true ribosomal constituents. If high molecular weight proteins are functionally associated with ribosomes, then these proteins can be expected to be present on ribosomes, solubilized from the membranes after removal of the supernatant fraction. If, on the other hand, these high molecular weight proteins are only adhering supernatant proteins, only the ribosomes from the supernatant are expected to contain such proteins.

In this communication we show that only the ribosomes from the supernatant fraction contain high molecular weight components, whereas the ribosomes from the membrane fraction contain no proteins larger than the 50S protein L2. We also show that polyribosome preparations may contain non-ribosomal proteins derived from subcellular structures, which sediment in a sucrose gradient in the same region as polyribosomes.

2. Experimental

2.1. *Isolation of ribosomes and flagella*

B. licheniformis (laboratory strain S244) was grown in continuous culture at 37°C in rich medium (13 g standard bouillon, 5 g yeast extract, 5 g bacteriological peptone and 5 g NaCl per liter) with a doubling time of 33 min and a dilution rate of 3 hr^{-1}. The cells were harvested at a density of 10^8 cells/ml. Lysis of the cells and separation of the ribosomal particles have been described in detail previously [5]. The 70S particles from the supernatant and the 70S particles and polyribosomes from the membrane fraction were collected by high speed centrifugation for 24 hr at 60 000 rev/min and 4°C in the 60 Ti rotor (Spinco). Polyribosomes were dissociated by suspending in buffer I (10 mM Tris–HCl, pH 7.6 at 4°C, 60 mM KCl and 0.3 mM magnesium acetate) and the derived subunits were separated by zonal centrifugation through 10–40% (w/v) sucrose gradients in the same buffer in a Spinco B XIV rotor. Subsequently, the separated subunits were collected by high speed centrifugation.

Flagella were prepared as described by Champness [6].

2.2. *Protein extraction and polyacrylamide gel electrophoresis*

The ribosome pellets were suspended in buffer II (10 mM Tris–HCl, pH 7.6 at 4°C, containing 60 mM

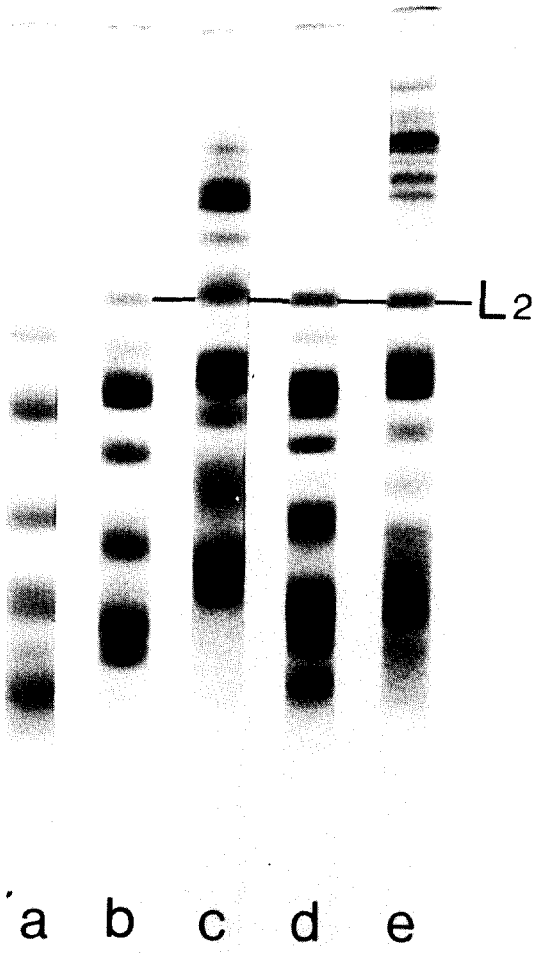

Fig.1. Electrophoretic analysis on SDS-urea gels of proteins from polysome-derived 30S (a) and 50S (b) subunits, 70S particles from the supernatant fraction (c), 70S particles from the membrane fraction (d) and *E. coli* 70S ribosomes (e). Our assumption that the largest 50S subunit protein in this analysis is identical to the protein L2 (nomenclature as in [10]) is based on its molecular weight, which was found by us to be 32, 000. This value is in close agreement with the previously published value of 32, 800 for L2 in *B. licheniformis* [11].

KCl, 10 mM magnesium acetate, 0.1 mM phenyl methyl sulfonyl fluoride (PMSF) and 6 mM 2-mercaptoethanol) and extraction of the ribosomal proteins was performed by the 2-chloroethanol method, described by Fogel and Sypherd [7]. In order to remove the 2-chloroethanol, the protein solution was dialyzed against distilled water, containing 1 mM HCl, 5 mM 2-mercaptoethanol and 0.1 mM PMSF. The same method was used for the preparation of flagelline.

E. coli 70S ribosomal protein was a gift from Dr J. A. Maassen (Laboratorium voor Fysiologische Scheikunde, Leiden, The Netherlands). The 70S ribosomes from *E. coli* MRE 600 were prepared according to Gesteland [8] and the ribosomal proteins were isolated using the acetic acid method described by Hardy et al. [1].

Electrophoresis of protein samples on SDS-urea gels was performed as described by Dunker and Rueckert [9]. The gels contained 10% (w/v) acrylamide and 0.34% (w/v) methylenebisacrylamide. For mol. wt determinations the following proteins were used as markers: catalase (60 000), ovalbumin (43 000), chymotrypsinogen (25 700) and cytochrome *c* (11 700). Electrophoresis on urea gels at pH 4.5 was performed following the procedure described by Hardy et al. [1]. The separation gel contained 15% (w/v) acrylamide and 0.3% (w/v) methylenebisacrylamide. The proteins were dissolved in 6 M urea, 10 mM HCl, 1 mM dithiothreitol.

For the designation of some of the ribosomal proteins (see figs 1 and 2) the nomenclature, introduced by Geisser et al. [10] was followed.

3. Results and discussion

The proteins of the ribosomes of both the membrane fraction and the supernatant fraction were analyzed by means of SDS gel electrophoresis. The results, together with the pattern for the proteins of *E. coli* 70S ribosomes, are shown in fig.1. The polysome-derived subunits and the 70S particles from the membrane fraction contain no proteins larger than the 50S protein L2. On the other hand, high molecular weight proteins are found on the ribosomes collected from the supernatant. These results support the suggestion [1–3] that the high molecular weight proteins on ribosomes are adhering supernatant proteins. That Subramanian did not detect these components in the post-ribosomal supernatant may be due to the fact that the proteins of this fraction were not extracted with acetic acid prior to electrophoretic analysis as was done with the ribosomal protein.

Another possible contamination of the ribosomal

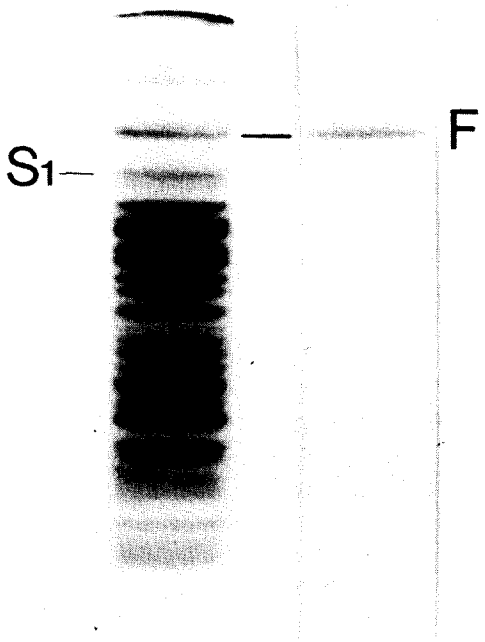

Fig.2. Electrophoretic analysis of proteins obtained from a polyribosome preparation (left) and the protein flagelline (right). The position of the 30S protein S1 (nomenclature as in ref. 10) has been verified in a separate analysis, using protein samples of the purified 30S and 50S subunits.

protein preparations may arise from subcellular structures cosedimenting with the ribosomal particles. This is illustrated by the analysis presented in fig.2. Most of the polyribosome preparations, isolated from the membrane fraction and purified by sucrose gradient centrifugation [5], did contain at least one protein component that was not found on one of the subunits. This component, designated F in fig.2, was present in varying amounts (from 1% to 15% of the total protein content of polyribosomes). In order to identify the origin of this protein electron micrographs were made of four independently prepared preparations of polyribosomes. The pictures obtained revealed that our polyribosome preparations contained, besides a very small proportion of membraneous material, varying amounts of flagella. Flagella sediment in the polyribosome region in sucrose gradients and are lost during the preparation of subunits from polyribosomes. We prepared pure flagella and found component F to be identical with the protein flagelline. The other slow moving components in the polysomal protein preparation (see fig.2) most probably represent membraneous material, which was always found in small amounts in the polysome preparations.

In summary, besides from the post-ribosomal supernatant, high molecular weight protein components in preparations of ribosomes may also derive from subcellular structures cosedimenting with the ribosomal particles.

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