PROTEIN COMPOSITION OF RIBOSOMES IN DNA-MEMBRANE COMPLEXES FROM BACILLUS LICHENIFORMIS

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SUMMARY. Ribosomes were isolated from DNA-membrane complexes prepared from Bacillus licheniformis using the "Sarkosyl M-band" technique (1,2). The protein composition of these ribosomes was analyzed by means of two-dimensional polyacrylamide gel electrophoresis. At least two ribosomal proteins were found to be absent from the 50S subunits of these ribosomes. As far as could be detected, no 30S subunit proteins were missing from these ribosomes.

INTRODUCTION

The structural heterogeneity of ribosomal subunits from E. coli has been studied extensively (3-6). Bickle et al. analyzed the protein composition of free ribosomal subunits and of subunits derived from polysomes, monosomes and single ribosomes (7). They found that three proteins were absent from one or more classes of subunits. We have been able to detect similar differences in the protein composition of free and polysome-derived subunits from B. licheniformis (manuscript in preparation).

Since in Bacillus species a fraction of the ribosomes is bound directly to the cytoplasmic membrane via the 50S subunit (2), we explored the possibility that structural heterogeneity of the 50S subunits might not only be related to the functional state of the subunits during the protein synthetic cycle, but also to the fact whether they are membrane-bound or not. To this end we isolated DNA-membrane complexes on a preparative scale using the Sarkosyl M-band technique (1,2). This method utilizes the selective adsorption of membrane components to crystals of magnesium-Sarkosyl and yields complexes which can be recovered as discrete bands ("M-bands") in sucrose density-gradients. Ribosomes are present in M-bands because

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they are attached either to membrane-bound DNA via messenger RNA or to the membrane directly (2).

We analyzed the protein composition of the membrane-bound ribosomes by means of two-dimensional polyacrylamide gel electrophoresis and found that the 50S ribosomal proteins L5 and L25a, and possibly also L9, L10a and L21, are not present on these ribosomes. The absence of these proteins is not due to the treatment with Sarkosyl since a similar treatment of total cellular ribosomes does not result in loss of any of the proteins mentioned.

MATERIALS AND METHODS

Preparation of ribosomes

B. licheniformis (laboratory strain S244) was grown and harvested as described previously (8). In order to convert the cells to protoplasts, 1 g of wet cells was homogenized in 18 ml of standard buffer (10 mM Tris-HCl, pH 7.6 at 4°C, 60 mM KCl, 10 mM magnesium acetate, 0.5 mM spermine, 0.75 mM spermidine, 1 mM dithiothreitol and 100 µg/ml chloramphenicol) containing 13% (w/v) sucrose and 0.015% (w/v) macaloid. To this suspension 2.5 ml of 0.3% (w/v) lysozyme in 0.25 M Tris-HCl, pH 7.6 at 4°C, 60 mM KCl, 10 mM magnesium acetate was added. The suspension was incubated during 10 min at 37°C and then cooled to 4°C. Half of the suspension of protoplasts (10 ml) was carefully mixed with 5 ml of a Sarkosyl solution (0.3% Sarkosyl in distilled water) and layered on a discontinuous sucrose gradient in TKM buffer (10 mM Tris-HCl, pH 7.6 at 4°C, 60 mM KCl, 10 mM magnesium acetate) containing 0.5 mM spermine and 0.75 mM spermidine. The discontinuous sucrose gradient consisted of a layer of 220 ml 47% (w/v) sucrose and a layer of 80 ml 20% (w/v) sucrose. After centrifugation in an International SW284 rotor during 5 hr at 2700 rev/min and 4°C, the M-bands were visible as white viscous bands at the interphase between the sucrose layers. M-bands of fifty gradients were collected. Cundliffe obtained M-bands containing only the membrane-bound ribosomes and 50S subunits by adding RNase to the lysate before centrifugation of the sucrose gradients (2). We were unable to obtain discrete bands at the interphase of the sucrose layers by this method, but observed granular material in both the interphase and the 20% sucrose layer. Therefore only Sarkosyl was added to the protoplasts for the preparation of the M-bands. M-bands prepared in this way contain about 20% of the total cellular ribosomal material (T.J. Stoof, personal communication).

In order to solubilize the ribosomes, DNase, Brij-58 and lipase were added to final concentrations of 2 µg/ml, 0.5% (w/v) and 65 µg/ml, respectively, and the suspension was incubated for 10 min at 37°C. Insoluble material was removed by centrifugation for 10 min at 10 000 x g and 4°C. The ribosomes were sedimented by centrifugation in a 60 Ti rotor (Spinco) for 24 hrs at 60 000 rev/min and 4°C, and suspended in TKM buffer. Samples of 0.5 ml, containing 1.8 mg of ribosomes were analyzed by layering them on linear 10-30% (w/v) sucrose gradients in TKM buffer followed by centrifugation in a SW27 rotor (Spinco) for 16 hr at 18 500 rev/min and 4°C. Gradient fractions containing 70S particles were

pooled and the ribosomes were collected by high-speed centrifugation. M-band 50S subunits were obtained in the same way. Total cellular 70S particles were prepared as described (8).

As a control we incubated total cellular 70S particles with Sarkosyl. Sarkosyl was added to a suspension of 4.9 mg of ribosomes in TKM buffer to a final concentration of 0.5%. After incubation for 10 min at 4°C, the ribosomes were centrifugated through sucrose gradients as described for the M-band ribosomes and collected by high-speed centrifugation.

Analysis of ribosomal proteins

Ribosome pellets were suspended in TKM buffer to a concentration of about 10 mg/ml and ribosomal protein was extracted with 2-chloroethanol, as described by Fogel and Sypherd (9). The ribosomal proteins were separated by two-dimensional gel electrophoresis according to Kaltschmidt and Wittmann(10). For the first dimension the 4% gel at pH 8.6 was used. A protein amount of about 750 µg in 9 M urea was applied per gel. No sample gels were used. The gel slabs were stained with Coomassie Brilliant Blue. The nomenclature, introduced by Geisser et al. (11) for the ribosomal proteins in Bacillus species is used throughout this paper.

RESULTS

The aim of this investigation was to compare the protein composition of total cellular ribosomes with that of M-band ribosomes. The most interesting fraction among the M-band ribosomes is that bound directly to the membrane via 50S subunits. Comparison of the protein composition of these subunits and other 50S subunits in the cell might reveal a relation between structural heterogeneity of the subunits and their physical state.

When the ribosomes were solubilized from the M-bands and separated by sucrose gradient centrifugation, we obtained a profile as shown in fig. 1. Very little or no material sedimenting faster than 70S particles is present. This observation was confirmed when preparations were centrifugated for shorter periods (results not shown). The 50S/30S subunit ratio in our preparations is in good agreement with the value obtained by Cundliffe for subunits in M-bands prepared from lysates treated with RNase (ref. 2, fig. 2). Cundliffe showed that ribosomes and 50S subunits present in such M-bands are membrane-bound. Thus, although we isolated M-bands in the absence of RNase, it appears that in our preparations we are dealing mainly with the membrane-bound fraction of the M-band ribosomes. A possible explanation for loss of DNA-bound ribosomes may lie in the fact that the protoplast suspension was mixed with Sarkosyl in a separate vial and not on the sucrose layers. Apparently most of the ribosomes which are bound to DNA via nascent

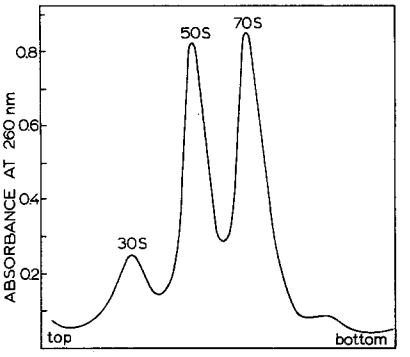


Fig. 1. Sucrose gradient analysis of the ribosomes isolated from M-bands.

mRNA are lost from the M-bands during the subsequent centrifugation step (see Materials and Methods).

The patterns, obtained upon two-dimensional polyacrylamide gel electrophoresis of proteins isolated from the total cellular 70S fraction and from the M-band 70S particles, are depicted in fig. 2. Comparison of these patterns shows that the 50S proteins L5, L9, L10a, L21 and L25a are virtually absent from the pattern obtained with ribosomes from the M-bands. Protein L2 seems to be present in reduced amounts on M-band ribosomes. No protein components can be observed in the pattern obtained with M-band ribosomes other than those normally found on 70S ribosomes. The absence of proteins L5, L9, L10a, L21 and L25a was also observed when M-band 50S subunits were analyzed. Since the 50S subunits represent that part of the ribosome interacting with the membrane (2) these results clearly suggest a relationship between structural heterogeneity of the ribosomes and their physical state. This heterogeneity appears to be limited to the 50S subunit. The patterns shown in fig. 2 do not indicate any difference in protein composition between 30S subunits from membrane bound ribosomes and 30S subunits from total cellular ribosomes. Such differences would be unexpected since 30S subunits do no play a role in direct binding of ribosomes to the membrane (2). The presence of a low amount of free 30S subunits in M-bands (fig. 1) is probably due to the presence of some DNA-bound ribosomes in our M-band preparations.

The lack of several proteins from M-band ribosomes could have been

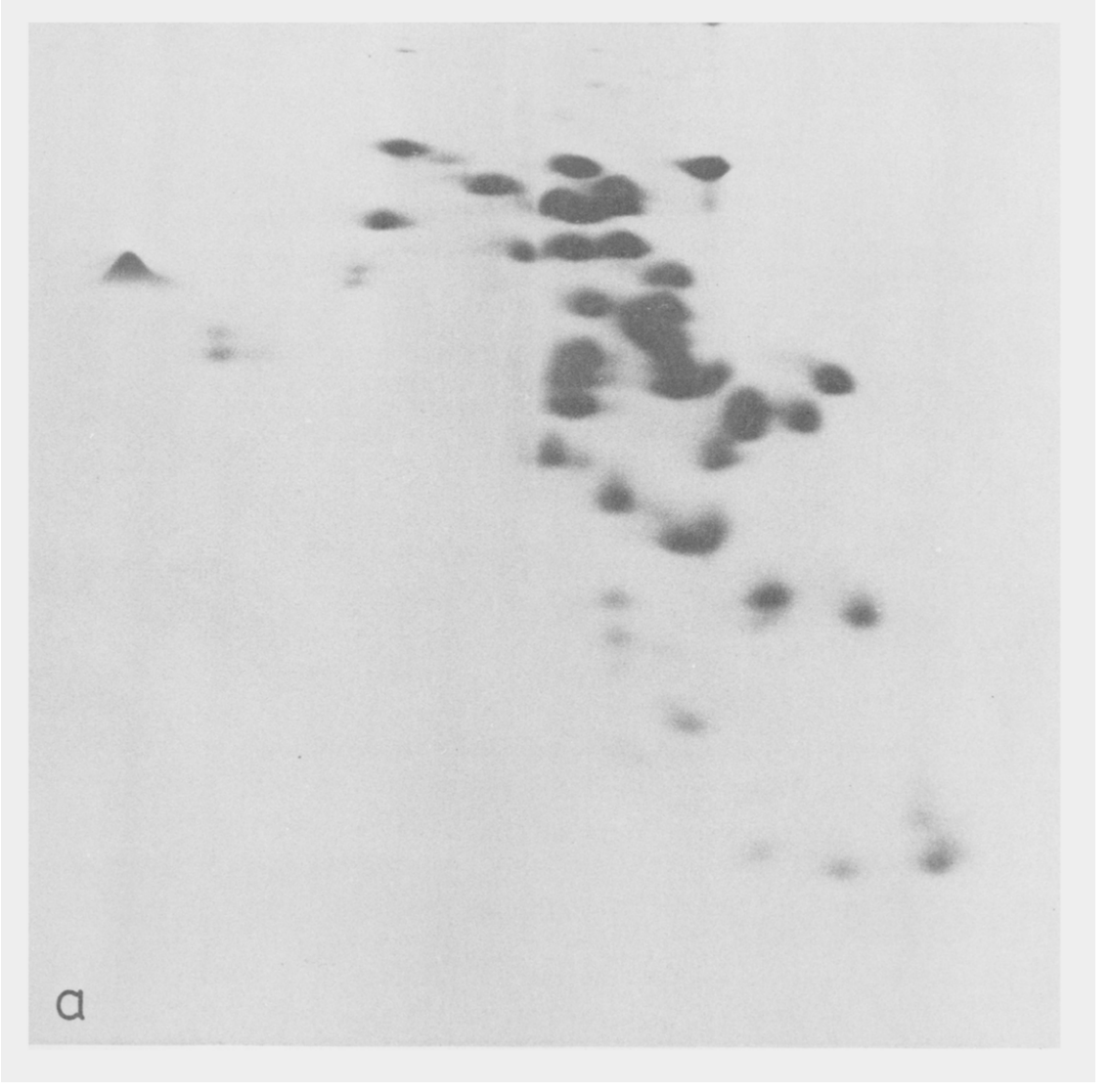
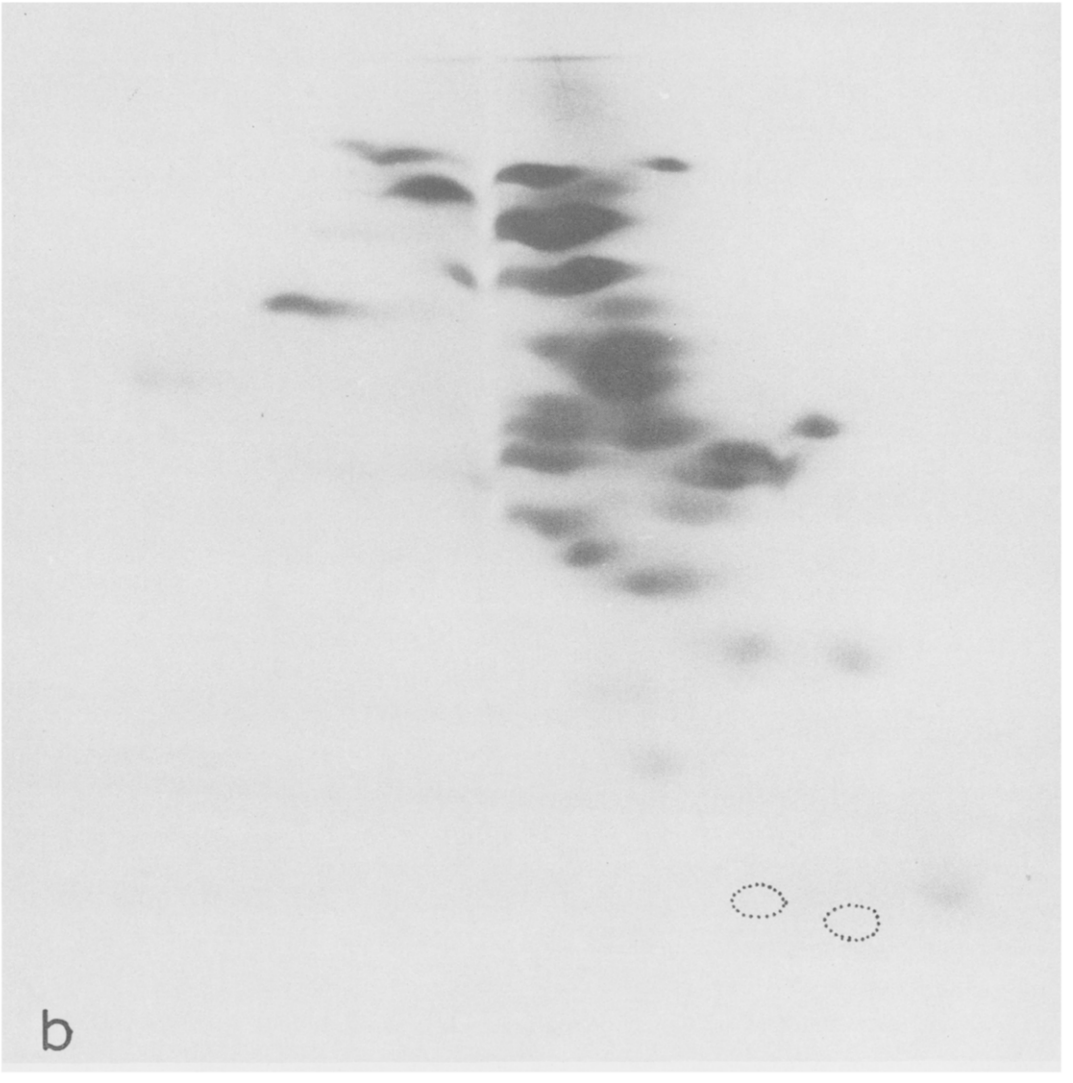


Fig. 2a-c. Two-dimensional electropherograms of proteins obtained from the total cellular 70S particles (a) and the M-band ribosomes (b). Fig. 2c shows a schematic representation of an electropherogram obtained with total cellular 70S ribosomal protein. Spots not found in the M-band ribosomal protein pattern have been cross hatched. Since no sample gel has been used protein L19 is only visible after electrophoresis in the first dimension during a longer period. Proteins S15, L29 and L30 are only faintly visible in the pattern obtained with M-band ribosomes (fig. 2b) and in most of the patterns obtained with total cellular ribosomes (cf. ref. 11, fig. 4a).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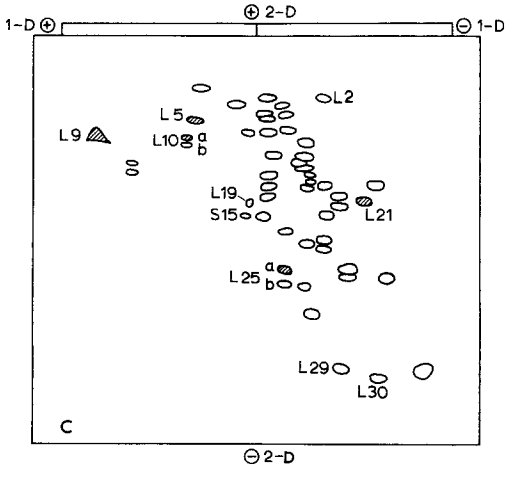
⊕ 2-D
1-D ⊕
⊖ 1-D
L2
L5
L9
L10 a
b
L19
S15
L21
L25 a
b
L29
L30
C
⊖ 2-D

caused by the treatment with Sarkosyl necessary to obtain M-bands. Therefore, as a control we analyzed Sarkosyl-treated total cellular 70S particles. The pattern obtained with the proteins from these ribosomes was qualitative and quantitative (as judged by the intensity of the stained spots) identical to that obtained with the proteins from untreated ribosomes (results not shown).

DISCUSSION

The results presented in this paper clearly show that structural differences exist between 50S subunits from membrane-bound ribosomes and 50S subunits from ribosomes not bound to membranes. Most significant is the absence of proteins L5 and L25a from membrane-bound ribosomes. These two proteins should be regarded as true ribosomal components, since they are not lost upon treatment with 1.0 M ammonium chloride (11). The low amounts of these two proteins still present in preparations of M-band ribosomes is probably due to the presence of a low amount of DNA-bound ribosomes in these preparations.

Although proteins L9, L10a and L21 also are virtually absent from M-band ribosomes, in these cases it is more difficult to decide whether this absence is related to the physical state of these ribosomes. Both protein L9 and L21 are loosely bound to the ribosome. L21 sometimes was found to be absent even from preparations of total cellular ribosomes or polyribosomes (see ref. 11, fig. 4a), while L9 is lost from 50S subunits upon dissociation at low magnesium ion concentrations (12). Although neither protein is lost upon treatment of total cellular ribosomes with Sarkosyl (which complexes with Mg^{2+} ions), we can not exclude the possibility of their loss during preparation of M-bands or subsequent operations.

Proteins L10a and L10b probably represent different forms of the same polypeptide, since in different experiments the intensity of one spot was inversely correlated with that of the other one. M-band ribosomes although lacking protein L10a do contain a relatively large amount of L10b. Apparently, protein L10 is present on the M-band ribosomes in only one form.

Because of these considerations, the question whether the absence of proteins L9, L10a and L21 is an artefact or a real property of M-band ribosomes can not be answered at the moment. As to proteins L5 and L25a, however, the situation is very clear. Both proteins are not

easily detached from the ribosomes and their absence from M-band ribosomes, thus, strongly suggests a difference in protein composition related to the physical state of these ribosomes.

Differences in protein composition between free and membrane-bound ribosomes have also been reported for other prokaryotic cells (13,14). In these cases, however, the proteins concerned could be removed from the ribosomes by washing with a buffer containing 1.0 M ammonium chloride (13,14) or by dissociation of the ribosomes (14). Therefore it is questionable whether these proteins should be considered as true ribosomal constituents.

Structural heterogeneity of ribosomal subunits related to their physical state has been described for a number of eukaryotic cells (15-18). The results, described in this paper, are the first unequivocal demonstration that this type of ribosomal heterogeneity also occurs in prokaryotic cells.

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