Correlation between the Rate of Exoprotein Synthesis and the Amount of the Multiprotein Complex on Membrane-bound Ribosomes (MBRP-Complex) in Staphylococcus aureus

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The membrane-bound ribosome protein (MBRP)-complex of Staphylococcus aureus was studied using antibodies to its individual components. The four polypeptides of the complex were firmly held together, and none were present in large excess. The membrane-bound fraction of the MBRP-complex was accessible to trypsin only after removal of the membrane-bound ribosomes; it also remained associated with the membrane-bound ribosomes even after solubilization of the membranes with Triton X-100. Furthermore, the amount of MBRP-complex in the membrane was proportional to the rate of exoprotein synthesis. These results strongly suggest a role for the MBRP-complex in protein secretion.

INTRODUCTION

In earlier work, aimed at identifying membrane proteins involved in protein secretion, membrane-bound ribosomes from Staphylococcus aureus were compared with ribosomes in the cytoplasm (Adler & Arvidson, 1984b). A protein of $M_r$ 60000 (MBRP-60) was found on ribosomes derived from the membrane but not on the cytoplasmic ribosomes. However, MBRP-60 was also found free in the cytoplasm, suggesting that this protein may cycle between the cytosol and the membrane. It was also found that antibodies against MBRP-60 precipitated this protein together with three other proteins with $M_r$ values of 71 000 (MBRP-71), 46000 (MBRP-46) and 41000 (MBRP-41).

In Bacillus subtilis, Marty-Mazars et al. (1983) identified a number of proteins which were present in ribosome-bearing domains of the membrane, but not in ribosome-free membranes. One of these proteins ($M_r$ 64000), which was also present in the cytoplasm (Horiuchi et al., 1983), was found to be antigenically related to MBRP-60 from S. aureus (Adler & Arvidson, 1984a), and appeared in a complex with three additional proteins (Adler & Arvidson, 1984a; Caulfield et al., 1984).

To shed further light on the function of the MBRP-complex its location in membrane–ribosome complexes was studied by determining the accessibility of the MBRP complex to trypsin. The distribution of the MBRP-complex between the membrane and the cytoplasm at different rates of exoprotein synthesis was also investigated.

METHODS

Bacterial strain and cultivation conditions. Staphylococcus aureus strain V8 was used throughout. Pre-cultures were grown in Trypticase Soy Broth (TSB, Difco) overnight on a rotary shaker. Bacteria from 10 ml of pre-culture were used to inoculate 50 ml of Brain Heart Infusion (BHI, Difco) medium in a 11 baffled shake flask. For radiolabelling experiments a defined amino acid medium (AA medium; Adler & Arvidson, 1984a) was used. Bacteria were harvested by pouring culture samples over 1 vol. of frozen buffer A (10 mm-Tris/HCl, 10 mm-

Abbreviations: MBRP, membrane-bound ribosome protein; TSB, Trypticase Soy Broth; BHI medium, Brain Heart Infusion medium; AA medium, amino acid medium; MDH, malic dehydrogenase; SDH, succinic dehydrogenase; SRP, signal recognition particle.
magnesium acetate, 50 mM-NH₄Cl, pH 7-4) containing 200 μg chloramphenicol ml⁻¹, followed by centrifugation at 4 ºC for 2 min at 15000 g.

Fractionation of bacteria. Preparation of protoplasts and fractionation of bacteria into membranes, cytoplasm, membrane ribosomes and cytoplasmic ribosomes were as described by Adler & Arvidson (1984b). Briefly, protoplasts were prepared by lysostaphin treatment in buffer A containing 1-1 M-sucrose. Osmostasis was in buffer A instead of phosphate buffer. After sonication for 2 × 10 s, the membranes were pelleted by centrifugation for 30 min at 27000 g. Cytoplasmic ribosomes were sedimented at 105000 g for 120 min. To detach ribosomes from the membranes, the membrane pellet was solubilized in buffer A with 4% (w/v) Triton X-100. The membrane ribosomes were then isolated by sucrose gradient (10–30%) centrifugation or by differential centrifugation (Adler & Arvidson, 1984b).

Quantification of membranes. Membranes prepared as described above were solubilized in buffer A with 4% Triton X-100, and the ribosomes were removed by centrifugation at 105000 g for 2 h. The supernatant was mixed with trichloroacetic acid and the precipitate assayed for protein (Peterson, 1977). This value was used to calculate the amounts of MBRP-complex per unit of membrane at different times during growth.

The quality of each membrane preparation was checked by measuring the malic dehydrogenase (MDH) and succinic dehydrogenase (SDH) activities (Adler & Arvidson, 1984b). The amount of MDH in the membrane preparation, which is a measure of cytoplasmic contamination, was always less than 10% of total activity in the protoplasts. More than 98% of the SDH activity in the protoplasts was found in the membrane preparations, indicating that most of the membranes were recovered by the centrifugation after osmolysis and sonicating of the protoplasts.

Determination of the rate of total exoprotein production. Bacterial dry wt and total extracellular protein concentrations were determined at intervals during growth in BHI. Total extracellular protein was measured by the method described by Abbas-Ali & Coleman (1977).

The specific rate of exoprotein production (Q) at different times of growth was calculated by the formula

\[ Q = \mu \frac{dP}{dx}, \]

where \( \mu \) is the specific growth rate constant and \( \frac{dP}{dx} \) is the differential rate of exoprotein production [i.e. the increase in exoprotein (P) per increase in bacterial mass (x)]. During the exponential growth phase \( \mu \) is constant (about 1·0 in BHI medium). However, during the post-exponential growth phase \( \mu \) gradually decreases. For this reason the mean growth rate constant (\( \bar{\mu} \)) was calculated for every 0·5 h interval by assuming an exponential increase in bacterial cell mass in each interval. The differential rate of exoprotein production was calculated from differential plots of extracellular protein concentrations versus bacterial dry wts.

Preparation of antibodies to the individual components of the MBRP-complex. MBRP-complex was isolated from Triton X-100 solubilized protoplasts (Adler & Arvidson, 1984b). The components of the complex were separated by SDS-PAGE (see below) and the individual bands were cut out from the gel, using Coomassie Brilliant Blue stained tracks as references. Extraction of proteins from the gel and immunization procedures were as described by Hedestedt & Rutberg (1980).

Immunoprecipitation of MBRP. Antibodies to the individual MBRP components were coupled to protein A-Sepharose CL-4B (Pharmacia) and used for immunoprecipitation of the MBRP-complex from various subcellular fractions (Adler & Arvidson, 1984a, b). Membrane fractions or protoplasts were solubilized in PBS (0-015 M-KH₂PO₄/NaH₂PO₄, 0-083 M-NaCl, pH 7-2) with 4% Triton X-100 and 1 μg DNase I ml⁻¹. Debris was removed by centrifugation at 10000 g for 20 min before immunoprecipitation. To immunoprecipitate the individual components the complex was dissociated by boiling in 1% (w/v) SDS according to Horiuchi et al. (1983), before addition of the antibodies.

Quantification of MBRP. The relative amounts of MBRP in different samples were determined by rocket immunoelectrophoresis (Adler & Arvidson, 1984b). Samples to be analysed were either solubilized in 4% Triton X-100 or by boiling in SDS (5 mg ml⁻¹). To the SDS-treated samples Triton X-100 was added to a final concentration of 25 mg ml⁻¹ before electrophoresis. MBRP-components were quantified by cutting out paper-copies of the rocket immunoprecipitates and weighing them. Samples to be compared were always run on the same gel.

Radiolabelling. To label bacterial proteins cells were grown for 5 h in AA-medium supplemented with 37 kBq [³⁵S]methionine ml⁻¹ (Amersham; >2·96 × 10⁶ GBq mmol⁻¹). To obtain satisfactory labelling of RNA, bacteria were pre-cultured in TSB overnight, and then shifted to fresh TSB for 1 h before inoculation in AA-medium containing 37 kBq [⁵,₆-³²P]uracil ml⁻¹ (Amersham; 1·5 × 10⁵ GBq mmol⁻¹). Bacteria were harvested during late post-exponential growth phase (5 h).

SDS-PAGE. This was done according to the methods described by Laemmli (1970), modified as described by Adler & Arvidson (1984b). Protein bands were visualized by staining with Coomassie Brilliant Blue. Radiolabelled proteins were detected by fluorography (Chamberlain, 1979).

Treatment of total cellular MBRP with proteinases. Protoplasts from [³⁵S]methionine-labelled bacteria were solubilized in PBS with 4% Triton X-100 to a final protein concentration of 1 mg ml⁻¹. Portions were kept for 60 min on ice or incubated at 37 ºC with different proteinases. The following enzymes were tested: trypsin, 2 mg ml⁻¹ (Sigma); protease K, 50 μg ml⁻¹ (Boehringer Mannheim); chymotrypsin, 0·1 mg ml⁻¹ (Worthington); subtilisin,
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0.1 mg ml⁻¹ (Novo Industri AB, Copenhagen, Denmark); and pronase, 0.1 mg ml⁻¹ (Boehringer Mannheim). At the end of incubation soybean trypsin inhibitor was added to the trypsinated samples to a final concentration of 2 mg ml⁻¹. To inhibit further activity of the other proteases phenylmethylsulphonyl fluoride was added to a final concentration of 1 mM. Remaining MBRP components were immunoprecipitated with the different MBRP antisera and analysed by SDS-PAGE.

Treatment of membrane-associated MBRP-complex with trypsin. Protoplasts from 5 ml of [³⁵S]methionine labelled culture were suspended in 1 ml of buffer A containing 1.1 M sucrose and 50 μg chloramphenicol ml⁻¹. Trypsin was added to a final concentration of 2 mg ml⁻¹ and the suspension was kept for 60 min on ice. Reaction was stopped by the addition of trypsin inhibitor to a final concentration of 2 mg ml⁻¹. Protoplasts were collected by centrifugation at 27000 g for 30 min and solubilized with Triton X-100 (4%, w/v) in PBS containing 2 μg DNase I ml⁻¹. MBRP components were immunoprecipitated and analysed by SDS-PAGE. As controls protoplasts were incubated with trypsin plus trypsin inhibitor. To let trypsin have access to the inner surface of the membrane radiolabelled protoplasts were osmolysed in the presence of trypsin. Protoplasts from 5 ml of culture were suspended in 1 ml of buffer A containing 2 mg trypsin, 50 μg chloramphenicol and 1 μg DNase I. After sonication for 2 x 10 s the lysate was kept on ice for 60 min. The reaction was stopped as above, the membranes were collected by centrifugation, and solubilized with Triton X-100 in PBS before immunoprecipitation and SDS-PAGE. Trypsin was also added to purified membrane preparations, which were analysed as described above.

Agarose gel electrophoresis of RNA. Total RNA was extracted from subcellular fractions with unbuffered phenol at 65 °C as described by Janson et al. (1986). The RNA was analysed by electrophoresis on horizontal agarose gel (1-2%, w/v, in 0.01 M-sodium phosphate buffer, pH 6.5).

Assays. Protein was determined by the Folin method. Samples containing SDS or Triton X-100 were treated as described by Peterson (1977) before assay. RNA was assayed according to Herbert et al. (1971). SDH and MDH, which were used as membrane and cytoplasmic markers respectively, were assayed as described by Adler & Arvidson (1984b).

RESULTS

Immunoprecipitation of the MBRP-complex with antibodies against the individual MBRP components

Each of the four antibodies to MBRP-71, MBRP-60, MBRP-46 and MBRP-41 precipitated the whole complex from radiolabelled protoplasts solubilized by Triton X-100 (Fig. 1a). Since it had been shown (Adler & Arvidson, 1984b) that the MBRP-complex was associated with membrane-bound ribosomes we would expect to find ribosomal proteins in the immunoprecipitates obtained from the Triton-solubilized protoplasts. However, the bond(s) between the ribosomes and the MBRP-complex seemed to require Mg²⁺ ions (see below) and were therefore disrupted during the immunoprecipitation and the subsequent washings, which were done in PBS. When buffer A was used instead of PBS considerable amounts of ribosomes were recovered in the immunoprecipitates. If the protoplasts were treated with SDS to dissociate the MBRP-complex before immunoprecipitation, only the single polypeptide which corresponded to the antibody used was seen (Fig. 1b).

As seen in Fig. 1a, the ratios between MBRP-71, MBRP-46 and MBRP-41 were approximately the same, irrespective of which antibody was used. The relative amounts of MBRP-60 varied between different experiments. This variation, which was independent of which antibody was used, indicates that MBRP-60 is more loosely bound to the complex than the other polypeptides, as noticed before (Adler & Arvidson, 1984b). The dissociation of MBRP-60 from the complex was most pronounced in preparations which had been frozen. Although there might be some uncomplexed MBRP-60 in some preparations, it can be concluded from these experiments that all the MBRP components were generally present in a complexed form.

Distribution of the MBRP-complex between subcellular fractions

During post-exponential growth phase MBRP-60 is present both on membrane-bound ribosomes (60%) and in the cytoplasm (40%) (Adler & Arvidson, 1984b). Since there is no major pool of uncomplexed MBRP proteins, the distribution of all four components of the MBRP-complex between subcellular fractions should be the same, and should also reflect the distribution of the entire complex. In fact, this was confirmed by quantitative immunoelectro-
Fig. 1. Autoradiogram of $[ ^{35} $S]methionine-labelled proteins immunoprecipitated with antisera raised to the different components of the MBRP-complex. The immunoprecipitates were separated by SDS-PAGE and autoradiographed. Samples were solubilized in 4% (w/v) Triton X-100 (a), or denatured by boiling in 1% (w/v) SDS (b), before immunoprecipitation.

phoresis of SDS-treated samples, using antibodies against the individual MBRP components (Table 1) and by immunoprecipitation followed by SDS-PAGE (Fig. 2). In several experiments the distribution of the MBRP-complex between the membrane and the cytoplasm was the same, indicating that our fractionation procedures were highly reproducible. Storage of the membranes at $ -70^\circ C $, or preparation of membranes from frozen protoplasts, resulted in decreased amounts of MBRP-complex in the membrane.

About 25% of the MBRP-complex in the cytoplasm was recovered with the ribosomes after centrifugation for 120 min at 105000 $g$. During sucrose gradient centrifugation the MBRP-complex closely followed the ribosomes, indicating that it was associated with them (Fig. 3a).
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Fig. 2. Autoradiogram showing immunoprecipitation of the MBRP-complex from different subcellular fractions. The samples were immunoprecipitated with MBRP-60 antiserum and subjected to SDS-PAGE. (a) Triton X-100 solubilized protoplasts; (b) isolated membrane ribosomes; (c) cytoplasmic ribosomes; (d) post-ribosomal cytoplasm.

Table 1. Distribution of the individual components of the MBRP-complex between the membranes and the cytoplasm

Samples were taken after 3 h growth in BHI medium separated into cytoplasm and membranes, and denatured by boiling in 1% (w/v) SDS. The relative amounts of the four MBRP components were determined by rocket immunoelectrophoresis against the individual antisera. The results shown are from a typical experiment.

<table>
<thead>
<tr>
<th>Content (%) of:</th>
<th>MBRP-71</th>
<th>MBRP-60</th>
<th>MBRP-46</th>
<th>MBRP-41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane</td>
<td>54</td>
<td>56</td>
<td>62</td>
<td>59</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>46</td>
<td>44</td>
<td>38</td>
<td>41</td>
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</table>

The sedimentation profile showed mainly 70S ribosomes. The reason why so few polysomes were seen was that these ribosomes had been sedimented once before, so that enough material could be applied on the sucrose gradient to allow the quantification of the MBRP-complex. The amount of MBRP per $A_{260}$ unit of ribosomes was only one-sixth that of membrane ribosomes. Since previously published data (Adler & Arvidson, 1984b; Fig. 5, panel b) indicated that there is only one MBRP-complex per membrane 70S ribosome it can be concluded that only a minor fraction (maximum about 15%) of the cytoplasmic ribosomes was associated with MBRP. The amount of MBRP per $A_{260}$ unit of membrane ribosomes was calculated from parallel experiments where membranes were solubilized with Triton X-100 and the ribosomes isolated by sucrose gradient centrifugation (Fig. 3b). These control experiments also showed that over 95% of the MBRP in the membrane sedimented together with the ribosomes, which means that MBRP in the membrane fraction is primarily associated with the ribosomes.
Release of MBRP-complex from membranes by exposure to low Mg\textsuperscript{2+}

When membranes prepared in the ribosome-preserving buffer A (10 mM-Mg\textsuperscript{2+}) were exposed to the same buffer with low Mg\textsuperscript{2+} (1 mM), 40–50\% of the ribosomes, measured as [\textsuperscript{3}H]uracil-labelled RNA, were released and remained in the supernatant after sedimentation of the membranes. Analysis of the RNA in the supernatant by agarose gel electrophoresis revealed the presence of roughly equal amounts of 23S and 16S RNA (data not shown), indicating that both the 30S and the 50S ribosomal subunits were released from the membrane at low Mg\textsuperscript{2+} concentration. This treatment also released 40–50\% (n = 5) of the membrane-associated MBRP-complex while the rest sedimented together with the membranes at 27000 g for 30 min. To determine whether the released MBRP-complex was still associated with the ribosomes, these were sedimented at 150000 g for 2 h. Only 30–50\% (n = 3) of the MBRP complex followed the ribosomes, indicating that the binding between the MBRP-complex and the ribosome is affected by the Mg\textsuperscript{2+} concentration, since ribosomes detached from the membranes with Triton X-100 at 10 mM-Mg\textsuperscript{2+} retained over 95\% of the MBRP-complex (see above). Consistent with previous results (Adler & Arvidson, 1984b), the MBRP-complex which sedimented together with the ribosomes after treatment with low Mg\textsuperscript{2+} was associated with the 50S ribosomal subunit as revealed by sucrose gradient centrifugation (data not shown).

The reason why only 40–50\% of the ribosomes appeared to be released from the membrane on exposure to low Mg\textsuperscript{2+} was that about half of the membranes were in the form of vesicles with the ribosomes attached to the inner surface. This was demonstrated by determination of the accessibility of SDH to trypsin. SDH is a membrane-bound enzyme which is only accessible on the cytoplasmic surface of the membrane, i.e. SDH was not affected when protoplasts were treated with trypsin.

Ribosomes which had been detached from the membrane by exposure to low Mg\textsuperscript{2+} but were trapped within membrane vesicles could partly (60–70\%) be released by sonication of the membranes. This treatment released very few ribosomes from membranes prepared at 10 mM-Mg\textsuperscript{2+}.

Accessibility of membrane-associated MBRP to trypsin

The sensitivity of Triton X-100 solubilized MBRP-complex to various proteinases was tested to find a suitable enzyme for studies of the accessibility of the complex when associated with membrane ribosomes. MBRP-71, MBRP-46 and MBRP-41 were all degraded by trypsin, chymotrypsin, proteinase K, subtilisin and pronase, whereas MBRP-60 was resistant to these
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Fig. 4. Autoradiogram showing the effects of trypsin treatment of membrane-associated MBRP-complex. After treatment the membrane fraction was pelleted and solubilized in PBS containing 4% (w/v) Triton X-100; the remaining MBRP-complex was immunoprecipitated with MBRP-60 antiserum. Lane (a), intact protoplasts in 1.1 M-sucrose in buffer A with trypsin; lane (b), protoplasts osmolysed in 10 mM-Mg^{2+} plus trypsin; lane (c), protoplasts osmolysed in 1 mM-Mg^{2+} plus trypsin.

enzymes. The most extensive degradation of the MBRP-complex was obtained with trypsin, which could also be efficiently inhibited by trypsin inhibitor. This enzyme was used for further experiments.

The accessibility of the MBRP proteins on the outer surface of the membrane was tested by incubating radiolabelled protoplasts with trypsin. After addition of trypsin inhibitor the protoplasts were osmolysed, and membranes were collected, solubilized with Triton X-100 and incubated with antibodies against MBRP-60. The immunoprecipitates were analysed by SDS-PAGE. This treatment did not alter any of the MBRP proteins, indicating that no trypsin-sensitive sequences were exposed on the outer surface of the cytoplasmic membrane (Fig. 4, lane a).

To test the accessibility of the MBRP-complex on the inner surface of the plasma membrane, protoplasts were lysed in the presence of trypsin. Control experiments, where the accessibility of SDH to trypsin was tested, revealed that the osmolysis procedures followed by a short sonication produced 60–70% of the membranes as correctly sealed vesicles and the remaining membranes as inside-out vesicles or open membrane fragments. SDH is bound to the inner surface of the plasma membrane (Hederstedt & Rutberg, 1981). Lysis of protoplasts in the presence of trypsin resulted in digestion of 80–85% of the SDH, indicating that most of the inner surface of the membrane was exposed to trypsin by this method. When protoplasts were lysed in buffer A (10 mM-Mg^{2+}) containing trypsin, there was no degradation of the MBRPs except for a slight decrease of MBRP-71 (Fig. 4, lane b).

The failure of the MBRPs to be digested with trypsin could be due to sensitive peptide bonds not being exposed on the surface of the membrane, or to protection by the ribosomes attached to the membrane. To test for the latter possibility protoplasts were osmolysed in buffer A with reduced Mg^{2+} concentration (1 mM). This treatment, which dissociated most of the ribosomes from the membrane (Adler & Arvidson, 1984b), increased the accessibility of the MBRPs dramatically. As shown in Fig. 4, lane (c), about 75% of MBRP-46 and MBRP-41 and almost all
Table 2. Correlation of membrane-associated MBRP-complex with the rate of exoprotein synthesis

The results are from the typical experiment represented in Fig. 5. The relative amounts of MBRP were determined in three sets of samples taken at the indicated times. The results are means ± SD.

<table>
<thead>
<tr>
<th>Cultivation time (h)</th>
<th>Specific rate of exoprotein synthesis [µg (mg dry wt)⁻¹ h⁻¹]</th>
<th>Amount of MBRP-complex (mg membrane protein)⁻¹</th>
<th>Amount of MBRP-complex (mg dry wt)⁻¹*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>3.3</td>
<td>0.27 ± 0.036</td>
<td>0.84 ± 0.056</td>
</tr>
<tr>
<td>1</td>
<td>3.2</td>
<td>0.33 ± 0.098</td>
<td>0.92 ± 0.080</td>
</tr>
<tr>
<td>2</td>
<td>5.7</td>
<td>0.73 ± 0.043</td>
<td>0.97 ± 0.063</td>
</tr>
<tr>
<td>3</td>
<td>10.4</td>
<td>1.00±</td>
<td>1.00†</td>
</tr>
</tbody>
</table>

* Washed cells (1.4 mg dry wt) were completely lysed with lysostaphin and Triton X-100 (4%, w/v) and used directly for rocket immunoelectrophoresis against MBRP-60 antiserum.
† Note that 60% of total cellular MBRP-complex is membrane bound after 3 h growth in BHI medium.

of MBRP-71 was degraded when trypsin was present in the osmolysis buffer. The same results were obtained when the ribosomes were detached from the membranes with EDTA. It is concluded that the membrane-associated portion of the MBRP-complex (with the possible exception of the protease-resistant MBRP-60) is covered by ribosomes and is therefore not accessible to trypsin.

Influence of the rate of exoprotein synthesis on the amount of membrane-associated MBRP-complex

If the MBRP-complex is functionally associated with exoprotein-synthesizing ribosomes then the amount of MBRP-complex in the membrane might reflect the rate of exoprotein synthesis.

The differential rate of exoprotein production of S. aureus is generally very low during the exponential growth phase and increases 5-10-fold during the post-exponential phase of growth (Abbas-Ali & Coleman, 1977). This pattern of exoprotein production was also seen with the strain used here (Fig. 5), and it has been shown (Janzon et al., 1986) that, for the exoproteins α-toxin and protein A, the increased production during post-exponential growth was due to an increased transcription of the relevant genes. The increase in total exoprotein synthesis during post-exponential growth was accompanied by a proportional increase in the amounts of MBRP-complex in the membrane (Table 2), strongly suggesting a role of the MBRP-complex in the
process of exoprotein translation and/or secretion. The finding that total cellular MBRP-complex was almost constant throughout the growth cycle (Table 2), indicates that MBRP-complex from the cytoplasmic pool was re-distributed to the membrane in response to the increased exoprotein synthesis/secretion.

Absence of RNA in the MBRP-complex

Since the MBRP-complex showed considerable similarities with the eukaryotic signal recognition particle (SRP) (Walter & Blobel, 1982) with respect to its cellular distribution and possible function, we examined the RNA content of the MBRP-complex. No radioactivity was immunoprecipitated together with the complex from \[\text{\textsuperscript{3}H}\text{uracil-labelled bacteria}, nor was the complex dissociated by extensive RNAase treatment.

DISCUSSION

By using antibodies against each of the individual components of the MBRP-complex we have been able to study the distribution of the complex and its components between different subcellular fractions. The complex, which seemed to be tightly held together, was found both on membrane ribosomes and in the cytoplasm (Table 1, Fig. 2).

Although we have not yet been able to demonstrate the exact function of the MBRP-complex the present data strongly suggest that it is involved in the process of exoprotein translation and/or secretion. The main indication of this was that the complex was protected from proteolysis by ribosomes on the inner surface of the membrane, suggesting that the complex is located between the ribosomes and the membrane. To make the MBRP-complex accessible to proteases the ribosomes were dissociated from the membrane by exposure to low Mg\(^{2+}\) concentration. That this treatment really detached the ribosomes from the membrane was carefully demonstrated in a series of control experiments, which also revealed that the entire MBRP-complex was released from the membrane together with the ribosomes. However, only about 50% of the MBRP-complex remained associated with the ribosomes, indicating that both the binding between the ribosomes and the membrane and that between the ribosome and the MBRP-complex require high Mg\(^{2+}\) concentrations. Similar results were obtained in B. subtilis, where ribosomes were released from the membrane at 0.01 mM-Mg\(^{2+}\) (Horiuchi et al., 1983), and where the S-complex could be released from the ribosomes by EDTA (Caulfield et al., 1984).

The location of the MBRP-complex between the ribosomes and the membrane is consistent with our previous finding that the complex is bound to the 50S subunit of the ribosome (Adler & Arvidson, 1984b), which is closest to the membrane, and which contains the nascent peptide exit site (Bernabeu & Lake, 1982). Since all membrane-associated MBRP-complex seemed to be bound to ribosomes (Fig. 3b), and because of its location between the ribosomes and the membrane, it is reasonable to believe that the complex is bound to the exoprotein-synthesizing ribosomes before they bind to the membrane. This is consistent with the presence of a pool of free complex in the cytoplasm, and with the appearance of a small number of MBRP-bearing ribosomes in the cytoplasm. In accordance with this theory it must be assumed that the MBRP-complex specifically recognizes exoprotein-synthesizing ribosomes, and that the binding of the complex to these ribosomes initiates some conformation change which allows the binding of the ribosome–MBRP-complex to the membrane. Despite the similarities between this model and the function of the eukaryotic SRP (Walter & Blobel, 1982), the MBRP-complex seems to be quite distinct from the SRP in that it contains only four polypeptides and no RNA.

Another important indication that the MBRP-complex is involved in the secretion of proteins comes from the demonstration that the amount of MBRPs in the membrane was proportional to the rate of exoprotein synthesis. From Table 2 it can also be concluded that the MBRP-complex is primarily involved in the synthesis/secretion of true extracellular proteins and not in the translocation of membrane proteins. If as little as 2% of total cellular proteins were membrane proteins that require the MBRP-complex for insertion into the membrane, this would completely mask the increase in membrane-associated MBRP-complex which was due to the increase in exoprotein synthesis. This was calculated in the following way. During the
exponential growth phase, when the growth rate constant was 1.0 h⁻¹ (Fig. 5), the specific rate of MBRP-requiring ‘membrane protein’ synthesis would be 8 µg (mg dry wt)⁻¹ h⁻¹, based on a total protein content in S. aureus of 40% (S. Arvidson, unpublished results). Together with the exoprotein synthesis during exponential growth phase (Table 2) this would give a total of 11 µg protein (mg dry wt)⁻¹ h⁻¹ that requires the MBRP-complex. During the late post-exponential growth phase (3 h) when the growth rate constant was only 0.1 h⁻¹, the rate of ‘membrane protein’ synthesis would be 0.8 µg (mg dry wt)⁻¹ h⁻¹, which, together with the exoprotein synthesis at this time (Table 2), makes 11.2 µg (mg dry wt)⁻¹ h⁻¹. Thus there would be no net increase in the amount of membrane-associated MBRP-complex.

Although exoproteins were primarily produced during the post-exponential growth phase the total amount of MBRP-complex was constant throughout the growth cycle. One possible explanation for the lack of correlation between synthesis of MBRP-complex and synthesis of exoproteins might be that the complex must be present at a high concentration to meet a sudden increase in exoprotein synthesis, as a result of nutrient limitation, and to prevent any accumulation of exoproteins in the cytoplasm. Among the extracellular proteins are several potent proteases, nucleases and phospholipases that would be harmful to the bacteria if present in the cytoplasm.

A complex of four polypeptides involved in the secretion of proteins from B. subtilis was identified by Caulfield et al. (1984). This complex, which was named the S-complex, is antigenically related to the MBRP-complex, and is composed of proteins of roughly the same Mₚ values as the MBRPs (Adler & Arvidson, 1984a). Although the MBRP-complex and the S-complex are obviously equivalent, different results have been obtained with respect to the location of the complexes. In S. aureus the entire complex seems to be covered by membrane-bound ribosomes, whereas in B. subtilis only one of the components (Mₚ, 64000, which is equivalent to MBRP-60) was protected from proteolysis by membrane-bound ribosomes (Caulfield et al., 1984). Furthermore, the B. subtilis S-complex was found to be associated with membrane-free ribosomes rather than with membranes. In the cytosol and in the membrane-ribosome fraction only the Mₚ, 64000 protein, without the other proteins of the complex, was found. Since the MBRP-complex has a tendency to dissociate, and MBRP-60 is more firmly bound to the ribosomes than the other proteins in the complex (Adler & Arvidson, 1984b), it is possible that the results obtained by Caulfield et al. (1984) are due to a similar instability of the S-complex. It should be noted that the preparation method used by Caulfield et al. (1984) involved several steps of centrifugation and storage of the membrane preparation in a frozen state.

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