Effect of Potassium Ions on the Attachment of Polyribosomes to the Membranes in Lysates of Exponential-Phase Cells of *Bacillus amyloliquefaciens*

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1. The distribution of ribosomal components between the soluble and membrane fractions of a preparation of exponential-phase cells of *Bacillus amyloliquefaciens* lysed with lysozyme in 0.05M-tris buffer, pH 7.6, containing 0.01M-Mg²⁺, was strongly influenced by the addition of K⁺ to the buffer, in the range 0–0.1M. 2. In the absence of K⁺, 37% of the ribosomal material was bound to the membrane and was not removed by repeated washing with the lysing buffer. The amount of bound material was progressively decreased on increasing the K⁺ concentration to 0.1M, when only 5% of ribosomal components remained attached to the membrane. 3. About 87% of the material that remained bound to the washed membranes prepared in the absence of added K⁺ was removed on suspension of the membranes in a buffer containing 0.1M-potassium chloride. 4. In the absence of K⁺, washed membranes, containing no detectable ribosomal material, were able to re-attach no more than half as much material as was found associated with membranes in the same buffer immediately after lysis. 5. There was no evidence of binding of specific components to the membrane. 6. In the presence of 0.05M-tris buffer, pH 7.6, maximum incorporation of amino acids into protein by *B. amyloliquefaciens* polyribosomes is effected in the presence of 0.01M-Mg²⁺ and 0.07–0.1M-K⁺, under which conditions less than 10% of the ribosomal material of a cell lysate would be membrane-bound.

It is postulated that membrane-bound polyribosomes are the major site of protein synthesis *in vivo* in bacteria and there is a considerable body of evidence supporting this idea. This evidence consists largely of the detection of membrane-associated ribosomes in cell lysates, the detection of labelled 'hot-trichloroacetic acid-insoluble' material in the membrane fraction of cells previously incubated with radioactive amino acids, and the demonstration that, *in vitro*, membrane-bound ribosomal material incorporated amino acids to a greater extent than did free ribosomal material. This work was reviewed by Hendler (1965).

The present paper is concerned with the nature of the attachment of ribosomal material to the cell membrane in bacterial lysates, that is not removed after repeated washing but is released by treatment with deoxycholate.

Aronson (1966) carried out the only detailed study on the nature of this attachment and he concluded that the presence of nascent polypeptides is the critical factor in the establishment of association with the membrane. Schlessinger, Marchesi & Kwan (1965) showed that Mg²⁺ had an effect on

the attachment; thus in the presence of 0.01M-tris buffer, pH 7.5, and 0.01M-sodium chloride the amount of membrane-bound RNA decreased from about 60% of the total cellular RNA at 0.02M-Mg²⁺ to 0% in the absence of Mg²⁺.

Consideration of all the data available reveals that little attention has been paid to the precise nature of the ionic environment in which cell extracts were prepared, particularly to the univalent cation present in the lysing medium. In this regard, during a study of amino acid incorporation by cell-free preparations of gently disrupted cells of *Bacillus amyloliquefaciens* the effect of different concentrations of Mg²⁺ and K⁺ was investigated, and it was found that in the presence of 0.05M-tris buffer, pH 7.6, and 0.01M-Mg²⁺ the distribution of ribosomal material between the soluble fraction and the membrane fraction was greatly influenced by the K⁺ concentration in the medium in which the cells were lysed, in the range 0–0.1M, whereas the distribution of ribosomal components in the two fractions was closely similar at every K⁺ concentration examined.

The results obtained are discussed in relation

to those of other workers, particularly with the closely related *Bacillus megaterium* system, and it is shown that the lack of agreement in the amount of membrane-bound ribosomes in the results reported by earlier workers can be explained if due attention is paid to the nature of the ionic environment.

The results of preliminary experiments were presented by Coleman (1968).

METHODS

Organism. The aerobic spore-forming bacillus used in this work was previously described as an unclassified strain of *Bacillus subtilis*; however, it was recently identified as *Bacillus amyloliquefaciens* strain T (Welker & Campbell, 1967).

Growth conditions. The organism was grown in a medium containing: $(\text{NH}_4)\text{H}_2\text{PO}_4$, 34 mM; KCl, 5 mM; MgSO_4 , 10 mM; CaCl_2 , 0.125 mM; ZnSO_4 , 0.0125 mM; sodium citrate, 2.125 mM; FeCl_3 , 0.5 mM; 'trace-metal ion' solution, 0.25 ml./l.; casein hydrolysate (Oxoid Ltd., London E.C.4), 0.5%; yeast extract (Oxoid Ltd.), 0.05%; maltose, 1%. The pH was adjusted to 7.2 with 10% (v/v) phosphoric acid.

The 'trace-metal ion' solution consisted of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (0.1 mg.), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (1.0 mg.), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.01 mg.) and ammonium molybdate (0.1 mg.) dissolved in 1 l. of water.

Batches (50 ml.) of the medium contained in 250 ml. conical flasks were inoculated, from a suspension of spores in water, by means of a platinum loop. The cultures were incubated for 19 hr. at 30° in a Gyrotory incubator shaker (model G 25; New Brunswick Scientific Co., New Brunswick, N.J., U.S.A.).

Preparation of cell fractions. Cells were harvested in the exponential phase, after incubation for 19 hr. at 30°, when $E_{600}^{1\text{cm}}$ of the tenfold-diluted culture was 0.250, by centrifuging the cultures for 2.5 min. at 5000g. The supernatant fractions were discarded and the cell pellets quickly frozen in liquid N_2 .

The frozen cells were then thawed in ice-cold TM buffer (0.05 M-tris-HCl-0.01 M-magnesium acetate, pH 7.6) containing KCl in the range 0–0.1 M, saturated with N_2 , and washed three times in the same buffer by centrifuging and resuspending.

Cells from each 50 ml. batch of culture were processed together, up to six different batches being treated at a time. Each preparation was washed in buffer containing a different KCl concentration and finally resuspended in a final volume of 5 ml. (ten times the original cell density) in the same buffer containing 2.5 mg. of crystalline lysozyme (British Drug Houses Ltd., Poole, Dorset) and 2.5 μg . of ribonuclease-free deoxyribonuclease (Worthington Biochemical Corp., Freehold, N.J., U.S.A.). The cell suspensions were flushed with N_2 and allowed to stand in tightly stoppered tubes at room temperature for 30 min., after which time cell lysis was complete.

Each cell lysate was then centrifuged for 5 min. at 5000g and the supernatant fraction was retained as a source of 'free' ribosomal material. The 'membrane' pellet was then washed five times, by centrifuging and resuspending, with batches (5 ml.) of ice-cold lysing buffer, after which no more 260 nm.-absorbing material was detected in the washings. The washed-membrane fraction was finally resuspended

to 5 ml. in lysing buffer, and 2.5 ml. of this suspension was retained for RNA assay. The other 2.5 ml. was centrifuged and the pellet was resuspended to 1.25 ml. in lysing buffer and aq. 10% (w/v) sodium deoxycholate (Koch-Light Laboratories Ltd., Colnbrook, Bucks.) added to give a final concentration of 0.2%. After being allowed to stand for 5 min. the preparation was centrifuged for 5 min. at 5000g and the supernatant fraction was retained as a source of 'membrane-bound' ribosomal material.

Re-attachment experiment. A series of membrane pellets from 2.5 ml. batches of lysate prepared in TM buffer containing 0.1 M-KCl were washed five times in 0.05 M-tris-HCl buffer, pH 7.6. The washed membranes were then resuspended to 2.5 ml. in TM buffer containing ribosomal material and the suspensions were incubated, with intermittent mixing, for 15 min. at room temperature. The membrane fractions were then isolated and washed five times in TM buffer, and finally resuspended to 1.25 ml. in TM buffer containing 0.2% (w/v) of sodium deoxycholate. After standing for 5 min. the preparations were centrifuged, and 0.4 ml. samples of the supernatant fractions were analysed on sucrose density gradients.

Sucrose-density-gradient analysis. The preparations containing the 'free' and 'membrane-bound' ribosomal material were analysed on sucrose density gradients after adjustment of their KCl content to 0.1 M by the addition of 2 M-KCl.

Samples of extract, 0.2 ml. of that containing the 'free' ribosomal material and 0.4 ml. of that containing the 'membrane-bound' material, were layered on to 5 ml. 15–30% sucrose gradients in TM buffer containing 0.1 M-KCl, and centrifuged in the SW 50L rotor of a Beckman Spinco L2–65 preparative ultracentrifuge at 45000 rev./min. for 60 min. to 10°.

The centrifuged gradients were analysed by monitoring them, at a rate of 0.5 ml./min., through an LKB Uvicord II u.v. flow analyser (fitted with a 2 mm.-light-path cell) coupled to a Photovolt Varicord model 43 log/linear recorder, as described by Coleman & Sykes (1966).

RNA assay. The RNA contents of cell fractions were determined by the orcinol method (Schneider, 1957). It was also possible to estimate the relative amounts of RNA in different fractions subjected to sucrose-density-gradient analysis, where the distribution of ribosomal components was similar, from the heights of the peaks in the density-gradient profiles.

RESULTS

Effect of K^+ concentration on distribution of ribosomal material. Cell lysates were prepared in media containing 0.05 M-tris-hydrochloric acid buffer, pH 7.6, and 0.01 M- Mg^{2+} (TM buffer) and different K^+ concentrations in the range 0–0.1 M. The lysates were then centrifuged and the soluble fractions retained as sources of free ribosomal material. The insoluble membrane fractions were washed by resuspension in buffer of the same composition as that in which the cells were lysed, until no more 260 nm.-absorbing material was eluted. The RNA content of each preparation in the series of soluble and washed-membrane fractions was then determined. In addition, alternate soluble fractions and

the material removed from the corresponding washed-membrane fractions by treatment with 0.2% deoxycholate were analysed on sucrose density gradients.

In Fig. 1 the effect of K^+ concentration on the distribution of RNA between the soluble and washed-membrane fractions is shown. In the absence of added K^+ there were equal amounts of RNA, in the soluble and washed-membrane fractions, each amounting to about 37% of the total RNA, with 27% of the total unaccounted for, which could, however, be recovered in the washings. On increase of the K^+ concentration there was a decrease in the amount of membrane-bound material and an increase in the RNA content of the soluble fraction, and also an increase in the recovery of material, until at 0.1 M- K^+ only 5% of the RNA was membrane-bound and a little over 85% was in the soluble fraction; at this K^+ concentration there was a 91% recovery of RNA.

Fig. 1 also shows the apparent distribution of material between soluble and washed-membrane fractions, i.e. the distribution calculated on the assumption that there was no loss of material during the washing of the membranes, 100% being taken as the sum of the material recovered in the soluble and in the washed-membrane fractions at each K^+

Fig. 1. Effect of K^+ concentration on the distribution of RNA between the soluble (○) and washed-membrane (●) fractions of a cell lysate. The experimental procedure is described in the Methods section. A correction was applied for the transfer RNA in the soluble fractions by subtracting from each value an amount equal to 10% of the total cellular RNA. Also shown (broken lines) is the 'apparent' distribution of RNA between the soluble (□) and washed-membrane (■) fractions, i.e. the values obtained by the assumption that there is no loss on washing.

concentration studied. In this case there was an apparent distribution of 50% in each fraction in the absence of added K^+ , decreasing to 5% in the membrane fraction and increasing to 95% in the soluble fraction at 0.1 M- K^+ .

The distribution of ribosomal components in the soluble and washed-membrane fractions at different

Fig. 2. Sucrose-density-gradient profiles of free and membrane-bound ribosomal material prepared (a) in the presence of 0.1 M-KCl, (b) in the presence of 0.04 M-KCl and (c) in the absence of KCl. The experimental procedure is described in the Methods section. In each case the upper profile shows the distribution of components in the soluble fraction and the lower profile shows the distribution in the washed-membrane fraction.

Table 1. *Distribution of ribosomal material between soluble and washed-membrane fractions of cell lysates*

The experimental procedure is described in the Methods section. The RNA in the soluble fractions, determined by the orcinol method, is corrected in each case for transfer RNA content, taken as 10% of the total cellular RNA, or 0.26 mg. The total material recovered at 0.1 M-KCl computed from sucrose-density-gradient profiles was taken as 100% in the absence of a value for total cellular RNA by this method.

Assay procedure	Concn. of KCl (M)	Ribosomal material			Percentage of material recovered	Percentage membrane-bound of total material	
		Soluble	Membrane-bound	Total recovered		Real	Apparent (assuming no loss on washing)
Computed from sucrose-density-gradient profile peak heights (expressed in arbitrary units)	0	1.56	1.62	3.18	68	34	51
	0.04	2.88	1.08	3.96	84	23	27
	0.10	4.20	0.69	4.89	100	14	14
Determined by RNA assay (expressed as mg. of RNA)	0	0.84	0.86	1.70	73	37	51
	0.04	1.54	0.39	1.93	83	17	20
	0.10	2.00	0.11	2.11	91	5	5

Fig. 3. Release of ribosomal material from washed membranes by the addition of 0.1 M-KCl. Washed membranes were prepared from three 2.5 ml. batches of washed-cell suspension lysed in 0.05 M-tris-HCl buffer, pH 7.6, containing 0.01 M-Mg²⁺, as described in the Methods section. The washed membranes were resuspended to 1.25 ml. in (a) 0.05 M-tris buffer, pH 7.6, containing 0.01 M-Mg²⁺, (b) as (a) with the addition of 0.1 M-KCl, and (c) as (a) with the addition of 0.2% (w/v) sodium deoxycholate. After standing for 5 min. the preparations were centrifuged at 5000g for 5 min. and 0.4 ml. samples of the supernatant fractions were analysed on sucrose density gradients.

K⁺ concentrations is shown in the sucrose-density-gradient profiles in Fig. 2. Apart from the low-molecular-weight material that remained at

the top of all the gradients on which soluble fractions were analysed and was absent from the material liberated from the membranes by deoxycholate, the profiles were closely similar in every case, with the 70s ribosomes as the largest single component and about half of the material sedimenting faster than 70s.

In Table 1 the relative amounts of material in the soluble and washed-membrane fractions, computed from the peak heights in the sucrose-density-gradient profiles, are shown and compared with the results of RNA assays at the same K⁺ concentrations. The two sets of results are similar, both showing maximum binding of ribosomal material in the absence of K⁺ of about 35%, and a 30% loss on washing; if this loss was ignored there was an apparent distribution with 50% of the ribosomal material in the membrane fraction.

The decreased recovery of ribosomal material on lowering of the K⁺ concentration is notable, since, if this simply represented loss of material occluded in the membrane pellet, then on washing the greatest loss might be expected at the higher K⁺ concentration where there was the largest amount of ribosomal material in the soluble fraction. A possible explanation for the observed characteristics might be that, as the difference between the ionic conditions within the intact cell and in the lysing medium increases in favour of binding, then particles may attach themselves to the membrane on lysis with varying degrees of firmness, so that a stable equilibrium between the various charged species might be expected to be established only after exhaustive washing, during which less firmly bound material is lost and only the most tightly bound ribosomal particles remain membrane-associated.

Removal of ribosomal material from washed membranes by increased K⁺ concentration. A cell

Fig. 4. Re-attachment of ribosomal material to washed membranes in the absence of added K^+ . This experiment was carried out as described in the Methods section. The washed membranes were incubated with (a) 0.05 M-tris-HCl buffer, pH 7.6, containing 0.01 M- Mg^{2+} (TM buffer) alone, (b) supernatant fraction from a preparation of cells at twice the normal concentration lysed in TM buffer, (c) a preparation obtained by centrifuging the soluble fraction from a cell suspension lysed in TM buffer containing 0.1 M-KCl at 150 000g for 60 min. at 0° and resuspending the ribosome pellet in TM buffer to the original volume, and (d) as (c) except that the ribosome pellet was resuspended to one-fifth of the original volume.

preparation was lysed in TM buffer and the membrane fraction washed five times with the same buffer, under which conditions there was maximum retention of ribosomal material on the membrane. The washed-membrane preparation was divided into three equal parts and each was resuspended to the same volume in TM buffer with no addition or with either 0.2% deoxycholate or 0.1 M- K^+ . After standing at room temperature for 5 min. the preparations were centrifuged and the supernatant fractions analysed on sucrose density gradients. The results in Fig. 3 show that, on resuspension in the washing buffer alone, a negligible amount of material was released. However, on addition of 0.1 M- K^+ a considerable amount of ribosomal material was liberated, and on solubilizing of the membrane with 0.2% deoxycholate an even larger amount of material was recovered in the soluble fraction. The relationship between the amounts of material liberated by 0.1 M- K^+ and by deoxycholate is consistent with the results shown in Figs. 1 and 2, where the 37% retention in the absence of K^+ was decreased to 5% with 0.1 M- K^+ and all 37% was liberated by 0.2% deoxycholate.

This liberation of ribosomal material from membranes on transfer from a buffer containing no K^+ to a more concentrated salt solution provides evidence that the observed effect of K^+ on the attachment is a real effect, and not a liberation of material from cells rendered fragile by the action of lysozyme that are then lysed in the presence of deoxycholate.

Re-attachment of ribosomal material to washed membranes in the absence of K^+ . This experiment was designed to test the ability of ribosomal material to attach itself to washed membranes containing no detectable ribonucleoprotein components. Two types of ribosomal preparation were tested: first, the soluble fraction of cells lysed in the absence of added K^+ , when maximum binding to membranes was achieved; secondly, a preparation from the soluble fraction of cells lysed in the presence of 0.1 M- K^+ , in which case about 85% of the total ribosomal material was in the soluble fraction. In the latter case, the soluble fraction was centrifuged at 150 000g for 60 min. and the ribosomes, which sedimented, were resuspended in TM buffer to about the same concentration as that in the original preparation, and further suspensions of the same material were prepared five and ten times as concentrated.

Fig. 4 shows the sucrose-density-gradient profiles of the material that was not removed from the membranes by repeated washing in TM buffer, but that was removed on solubilizing the membranes with deoxycholate. Material was re-attached to the membrane, and there was little difference in the amounts attached in either of the two preparations containing ribosomes at the same concentration. In both cases the amount attached corresponds to about 30% of that found to be membrane-associated in a cell preparation lysed in TM buffer. This suggests that there is no specific binding of certain components to the membrane when cells are lysed

in the absence of added K^+ . Increasing the concentration of ribosomes fivefold resulted in increased attachment, to about 50% of that in the washed-membrane fraction of cells lysed in the absence of added K^+ . No further increase in attachment was observed on increasing the concentration of the ribosomes to ten times the lowest concentration tested.

The distribution of ribosomal components in all the profiles was similar, and they show loss of polysomes when compared with the profiles in Fig. 2. However, in view of the conditions of the re-attachment procedure this is understandable, and an examination of the distribution of components in the ribosomal suspension before and after the incubation with membrane showed no selective loss of any component.

The decreased binding of ribosomes observed when washed membranes were treated with a suspension of free ribosomal material, compared with the attachment observed immediately on cell lysis, is not surprising, since it might be expected that when cells are lysed a high concentration of ribosomes is in contact with extended membranes, whereas in re-attachment experiments the membrane might be expected to present itself in a less favourable manner, because of the collapse of cell envelopes, the aggregation of material and the formation of intermembrane Mg^{2+} bridges stable to washing. Nevertheless, the above experiment does confirm that attachment of ribosomal material to bacterial membranes is dependent on the ionic environment.

DISCUSSION

These results are consistent with Mg^{2+} binding ribosomes to the membrane by forming bridges between the negatively charged groups on these two components. The addition of univalent cation, in this case K^+ , would effect a screening of the negative charges, thus preventing the formation of Mg^{2+} bridges and so increasing the amount of free-ribosomal material. This is in keeping with the work of Schlessinger *et al.* (1965), mentioned above, indicating that lowering of the Mg^{2+} concentration results in a loss of membrane-bound ribosomal material. The effect of univalent cation on the binding of ribosomes to membranes was recognized by Littlefield & Keller (1957), who found that ribosomes that could be released from mouse Ehrlich ascites-tumour cell microsomes by deoxycholate, which was found to inhibit amino acid incorporation, could also be liberated by a high concentration of sodium chloride, and this was achieved by homogenizing with 0.5M-sodium chloride, 5mM-magnesium chloride and 0.01M-tris buffer, pH 7.6.

Table 2 summarizes the results obtained by various investigators who have studied *B. megaterium* and shows the percentage of ribosomal material that they found to be membrane-bound, in relation to the ionic conditions during cell lysis. Godson, Hunter & Butler (1961) were among the earliest supporters of the idea that bacterial protein synthesis occurs on the membrane. They usually lysed protoplasts in phosphate buffer at a concentration that would precipitate all the Mg^{2+} present, and found that 50% of the RNA was membrane-bound, usually as 30s and 50s particles, which is consistent with non-availability of Mg^{2+} . However, this 50% was readily removed by washing, and there is some suggestion from their electron-microscopic data that vesicles exist in their preparations, which would accord with the ease of removal of ribosomal material by washing. This therefore does not constitute convincing evidence that polysomes are membrane-bound.

Schlessinger (1963) and Schlessinger *et al.* (1965) found that 33–50% of the ribosomal material was membrane-bound in the presence of 0.01M- Mg^{2+} and 0.01M- Na^+ , but was lost on removing Mg^{2+} ; this is consistent with the results shown in Fig. 1. They studied cell-free incorporation at 0.04M- K^+ , although there are no data to suggest the reason for this change in concentration of cation.

Aronson (1966) lysed cells in the presence of 0.01M- Mg^{2+} and 0.01M- K^+ , under which conditions about 30% of the ribosomes might be expected to be membrane-bound; however, he washed the membranes with water and found little RNA associated with the membrane, which would be expected. In further experiments with isolated membrane and polysomes he demonstrated a 42% attachment of exponential-phase polysomes in the presence of the 0.01M- Mg^{2+} -0.01M- K^+ buffer.

Yudkin & Davis (1965) found 25% of the RNA bound, which they detected as 23s and 16s ribosomal RNA after treatment of washed membranes with sodium dodecyl sulphate followed by phenol extraction. In this case, therefore, the precise nature of the bound ribosomes is not known. A 25% attachment of ribosomes to the membrane at a decreased Mg^{2+} concentration (2mM) and no univalent cation is not inconsistent with the data expressed in Fig. 1 in conjunction with the effect of Mg^{2+} reported by Schlessinger *et al.* (1965), but the reason why six washings with 0.01M-tris buffer alone do not remove this material is not explained.

Weibull (1953) extracted with phosphate buffer with no Mg^{2+} present, and Schaechter (1963) disrupted cells in 0.01M- Mg^{2+} and 0.1M- K^+ ; both found little or no membrane-associated RNA, as would be expected.

The same pattern of attachment of ribosomes to membranes is found with systems other than

Table 2. Summary of data obtained from literature of studies on *B. megaterium*

Reference	Conditions of lysis	RNA membrane-bound (%)	Remarks
Godson <i>et al.</i> (1961)	0.2 M-Phosphate-salts, pH 7.0, with 4 mM-Mg ²⁺ or 0.6 M-tris	~50	95% lost on washing with 0.03 M-phosphate-salts or 0.01 M-tris, each containing 0.01 M-Mg ²⁺
Schlessinger (1963); Schlessinger <i>et al.</i> (1965)	0.01 M-Tris-HCl, pH 7.5, 0.01 M-Mg ²⁺ , 0.01 M-NaCl (0.01 M- β -mercaptoethanol)	33-50	All lost on lowering Mg ²⁺ concentration to zero; cell-free incorporation in 0.04 M-KCl-9 mM-Mg ²⁺ -5 mM-tris-HCl, pH 7.3
Aronson (1966)	0.01 M-Tris-HCl, pH 7.4, 0.01 M-Mg ²⁺ , 0.01 M-KCl	42	Little RNA membrane-bound after five or six washings with water; resuspended membranes and isolated polysomes (exponential phase) in lysing buffer, 42% bound
Yudkin & Davis (1965)	2 mM-Tris-HCl, pH 7.2, 2 mM-Mg ²⁺	25	Not removed on washing with 0.01 M-tris-HCl, pH 7.2
Weibull (1953)	0.03 M-Phosphate, pH 7.0	15	
Schaechter (1963)	0.01 M-Tris-HCl, pH 7.0 0.01 M-Mg ²⁺ , 0.1 M-KCl	Trace	

B. megaterium. Thus Hendler & Tani (1964) found that the distribution of hot-trichloroacetic acid-insoluble radioactivity between soluble and membrane fractions of *Escherichia coli* cells, labelled with ¹⁴C-labelled amino acid before disruption of the cells, was dependent on the method of cell breakage. However, on disrupting the cells in 1 mM-Mg²⁺-5 mM-tris buffer, pH 8, about 10% of the RNA was membrane-bound, consistent with the low Mg²⁺-low binding idea (Schlessinger *et al.* 1965).

Moore & Umbreit (1965) found 11.6% of the RNA to be membrane-bound in preparations of *Streptococcus faecalis* lysed in the presence of 0.12 M-K⁺ and 0.01 M-Mg²⁺, as might be expected. Gilby, Few & McQuillen (1958) lysed *Micrococcus lysodeikticus* in 0.1 M-sodium chloride, washed the preparation three times with 0.05 M-sodium chloride and finally resuspended it in 0.05 M-sodium chloride, and found no more than a trace of nucleic acid in the membrane fraction.

It is clear from the above results that a suitable choice of ionic environment will cause the binding of more or less ribosomal material to the cell membrane, and to establish what proportion is membrane-bound in bacterial cells *in vivo* would require more detailed information than is available on the precise nature of the intracellular environment; but in the absence of this knowledge, and if such structures are membrane-bound for a valid physiological reason, then probably the best and only way we might detect them is to lyse the cells, and treat the membranes, as gently as possible, i.e. under conditions known to preserve maximal cell-free protein-synthesizing capacity. In the presence of 0.05 M-tris buffer, pH 7.6, maximum amino acid incorporation into protein by preparations of gently disrupted cells of *B. amyloliquefaciens*

is achieved in the presence of 0.01 M-Mg²⁺ and 0.07-0.1 M-K⁺ (Coleman, 1969), under which conditions less than 10% of the ribosomal material of a cell lysate was membrane-bound. Therefore, according to the above argument, this small membrane-bound fraction may have a specific role in bacterial macromolecule synthesis, but, on the other hand, it must be emphasized that this situation does not necessarily bear any relationship to that in intact cells.

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