12-1971

# Fraction of Bacterial Ribosomes

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# FRACTIONATION OF BACTERIAL RIBOSOMES

by

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A Thesis
Submitted to the
Faculty of the Graduate College
in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
December 1971

ACKNOWLEDGEMENTS

The author wishes to express her most sincere appreciation to Dr. Jochanan Stenesh for his wisdom, extreme understanding and never ending patience which made the completion of this research possible.

Acknowledgement is also given to my husband Kung-Wei for his patience and encouragement.

Katty Yang

PLEASE NOTE:

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# INTRODUCTION

Ribosomes are subcellular particles occurring predominantly in the cytoplasm. They are macromolecules composed of protein and ribonucleic acid (RNA) and are the site where protein synthesis takes place. In animal and plant cells, the ribosomes are attached to the endoplasmic reticulum and have a sedimentation coefficient of 80S (1). In bacteria, they are free and have a sedimentation coefficient of 70S (1).

Ribosomes are made up of two subunits. In bacteria, the subunits have a sedimentation coefficient of 50S and 30S and a molecular weight of approximately $2x10^6$ and $1x10^6$, respectively (2). The 50S subunit is slightly dome-shaped and has dimensions of about 160x160x130 Å. The 30S subunit is more flattened and has dimensions of about 70x180x180 Å. In the 70S monomer, the smaller subunit fits like a cap onto the flattened surface of the 50S subunit (3). The monomeric ribosomes can also associate to form dimers (100S) and higher aggregates.

Bacterial ribosomes contain 40-65% RNA and 60-35% protein with little or no lipid. The 50S subunit contains two RNA molecules (23S,5S) and some 30 different proteins. The 30S subunit contains one RNA molecule (6S) and about 20 different proteins. The ribosomal RNA is

rich in purine nucleotides, and the ribosomal proteins, are very heterogeneous in molecular weight (about 10,000-40,000).

The stability of the 70S ribosome is linked to its involvement in protein synthesis. At the start of protein synthesis, a 30S and a 50S subunit join together with messengerRNA (mRNA) and formylmethionyl-transfer RNA (tRNA) to form the 70S "active ribosome." The ribosome then moves across the mRNA as the protein is synthesized, and dissociates into 30S and 50S subunits when synthesis is completed (4,5). The mRNA binding center and the tRNA binding site are thought to be in the contact region between the two subunits (6,7). In protein synthesis, the association of 30S and 50S subunits and the dissociation of 70S monomers require specific initiation and dissociation factors (8,9). The stability of the 70S ribosome also depends on the presence of mRNA, aminoacyl-tRNA and peptidyl-tRNA. In the case of Escherichia coli, free 30S and 50S subunits associate at high $Mg^{++}$ concentrations but the 70S monomers formed are unstable at a $Mg^{++}$ concentration of 0.005M. If the 30S subunit is bound to aminoacyl-tRNA and mRNA, then the 70S monomer formed with free 50S subunits is stable at 0.005M $Mg^{++}$, but dissociates completely at 0.001 M $Mg^{++}$. The presence of peptidyl-tRNA on the 50S subunit stabilizes the 70S to a similar extent. Intact 70S ribosomes

are more stable and dissociate only at $Mg^{++}$ concentrations below 0.001M (10).

Ribosome stability and conformation are also dependent on the ratio of monovalent to divalent cation concentrations R (11). At R values between 0.4 and 10, the ribosome exists in a rather compact form. As R is increased above 10, the ribosome loosens up and ultimately dissociates into 30S and 50S subunits. This seems to indicate a competitive relationship between mono- and divalent cations.

It is clear from the foregoing that the basic ribosomal unit in bacterial protein synthesis is the 70S monomer. To study it properly, it is important to obtain it in purfied form, devoid of subunits and larger aggregates. It is with this in mind that the present research was undertaken. Basically, three approaches were used to obtain purified 70S preparations.

The first approach involved the use of sucrose density gradient centrafugation which had been used successfully for the fractionation of ribosomes and subunits from E. coli (2,12) and guinea pig (13).

The second approach consisted of variations in the method of ribosome isolation and in the make up of the final suspending medium.

The third approach, and the most successful one, involved the fractionation of ribosomes by gel filtration.

Heretofore, only a preliminary study had been made of this method by separating 50S subunits from large aggregates in the case of E. coli (14).

The mesophile Bacillus licheniformis was chosen for this study since previous work had resulted in a ribosomal preparation which consisted predominantly of 70S ribosomes (15,16).

## MATERIALS AND METHODS

### Organism and Growth Conditions

A mesophilic strain of the genus Bacillus, B. licheniformis NRS 243 was used throughout. Growth of the cells for Parts I-II was on petri dishes (15,16), and for Part III was by means of a fermentor (17).

### Isolation of Ribosomes

The ribosomes were prepared by two procedures; one procedure was used for Parts I and II and the other procedure was used for Part III. These procedures have been described previously in reference (15,16) and (17), respectively. Any variations from these procedures are indicated in the text.

### Apparatus

For low speed centrifugations up to 12,000xg, a Sorvall model RC-2 refrigerated centrifuge was used; for high speed centrifugation above 12,000xg, a Spinco model L preparative ultracentrifuge was used. All analytical ultracentrifuge studies were made at 60,000xg in a Spinco model E analytical ultracentrifuge, equipped with rotor temperature indicator control (6.6° - 10.8°C) and with schieren and ultraviolet optics.

Measurements of the photographic plates and U.V. films were made with a Nikon model 6C microcomparator and a Joyce-Loebl Chromoscan Densitometer, respectively.

Spectrophotometric measurements were made with a Zeiss model PMQ-II spectrophotometer.

Area measurements of enlarged ultracentrifuge patterns were made with a Gelman model 139231 planimeter.

Sucrose density gradients were formed and the tubes were punctured using a Buchler Instruments apparatus.

Gel filtration was carried out using a 2.5x100 cm column (Pharmacia Fine Chemicals). Generally, one flow adaptor was used with the column.

## Reagents

Ribonuclease-free sucrose was purchased from Mann Laboratories and BioGel A-15m (100-200 mesh) was purchased from BioRad. Tris (hydroxymethyl)-aminomethane, Tris, was obtained from Sigma and magnesium acetate was obtained from Matheson. Alumina was a chromotographic grade (Merck 71707) and deoxyribonuclease was obtained from Worthington Biochemical Corporation.

Other reagent grade chemicals used were: spermidine, 2-mercaptoethanol, potassium chloride, calcium chloride and ammonium chloride.

Buffer A (0.005M Tris, 0.005M magnesium acetate, pH 7.4) was used in density gradient centrifugation.

Buffer B (0.01M Tris, 0.01M magnesium acetate and 0.06M ammonium chloride, pH 7.4) was used in gel filtration chromotography and 0.006M spermidine and 0.006M 2-mercaptoethanol were added to buffer B just before use. Buffer C (0.005M Tris and 0.002M magnesium acetate, pH 7.4) was also used in gel filtration chromotography. The buffer used for Part II are described in the text.

## Density Gradient Centrifugation

A linear (5-20%) density gradient was formed using 2.6 ml of 5% (w/v) sucrose and 2.4 ml of 20% (w/v) sucrose. The ribosome solution (0.1 ml) was applied to the gradient with a pipette. The tubes were centrifuged at 50,000xg for 150 minutes in a swinging bucket rotor (SW-39) in the Spinco ultracentrifuge (Model L). The rotor was allowed to coast to rest without braking. The tubes were punctured, and 10 drop fractions were collected manually (5.1 ml yielded about 35 fractions). Distilled water (3 ml) was added to each fraction and the absorbance of the solution was then measured at 260 nm.

## Variation in Ribosome Preparation

Different buffers, as indicated in Results and Discussion, were used to dissolve the ribosomal pellet obtained from the first high speed centrifugation (Spinco Model L). The cycles of low and high speed centrifugation

(15,16) were then repeated twice with the particular buffer. For the potassium chloride wash experiment, buffer containing potassium chloride was used to dissolve only the pellet from the first high speed centrifugation; the remaining two cycles of low and high speed centrifugation involved buffer without potassium chloride.

## Gel Filtration Chromotography

The column 2.5x100 cm was packed by filling half of the column with buffer and then adding the diluted gel (1 liter of gel and about 500 to 1000 ml of buffer) using a wide-stem funnel. The column outlet was opened as the bed formed and the pressure head was maintained at 5 to 10 cm. The gel was stabilized by passing the appropriate buffer through the column overnight. About 0.4-0.8 ml of ribosome solution (absorbance of about 300 at 260 nm) was layered onto the column with a pipette or was injected into the capillary tubing of the flow-adaptor with a hypodermic syringe. The flow-adaptor was used to eliminate the dead volume and to prevent the expansion of the gel. The buffer reservoir was then connected to the column and the ribosomes eluted with the appropriate buffer. The buffer reservoir was a Mariotte type bottle which is a bottle with an outlet at the bottom and a pressure regulator. The latter is simply a piece of glass tubing which extends through a rubber stopper to

slightly above the outlet of the bottle. In this fashion, the hydrostatic pressure head on the column can be kept constant even though the buffer volume in the reservoir decreases with time. The pressure was generally maintained at about 75 cm. Fractions were collected at two minute intervals using a flow rate of about 30 ml/hr. These operations were performed at $4^{o}C$. Each fraction was diluted with 5 ml of distilled water and the absorbance was then measured at 260 nm.

For some experiments, the ribosomes, after having been isolated in buffer B, were dialyzed versus buffer C before being applied to the column.

## RESULTS AND DISCUSSIONS

### Density Gradient Centrifugation

Sucrose density gradients were prepared and analyzed as described under Methods. The linearity of the gradient was checked by collecting fractions and measuring the refractive index with an Abbe refractometer. The results are shown in Figure 1 where it can be seen that the gradient was linear and could be formed in a reproducible manner.

The sedimentation coefficient ($S^{0}_{20,w}$) of the major components in the final ribosome preparation were 28, 47, 63 and 113S. At infinite dilution these correspond to the standard 30S, 50S, 70S and 100S bacterial ribosomes. The analytical ultracentrifuge pattern of this ribosome preparation is shown in Figure 2. When this ribosome preparation was analyzed by sucrose density gradient centrifugation, the pattern shown in Figure 3 was obtained. It can be seen that the peak fractions were not as well separated by sucrose density gradient centrifugation as by analytical ultracentrifugation. The density gradient separations were, however, reproducible, and were shifted toward the less dense part of the gradient as the ribosome concentration was decreased. This might be due to a slight decrease in ribosome density

Figure 1. Refractive Index of a Linear Sucrose Density Gradient.

△,● - Sucrose density gradients before centrifugation.

○ - Sucrose density gradient after centrifugation for 150 minutes at 50,000xg.

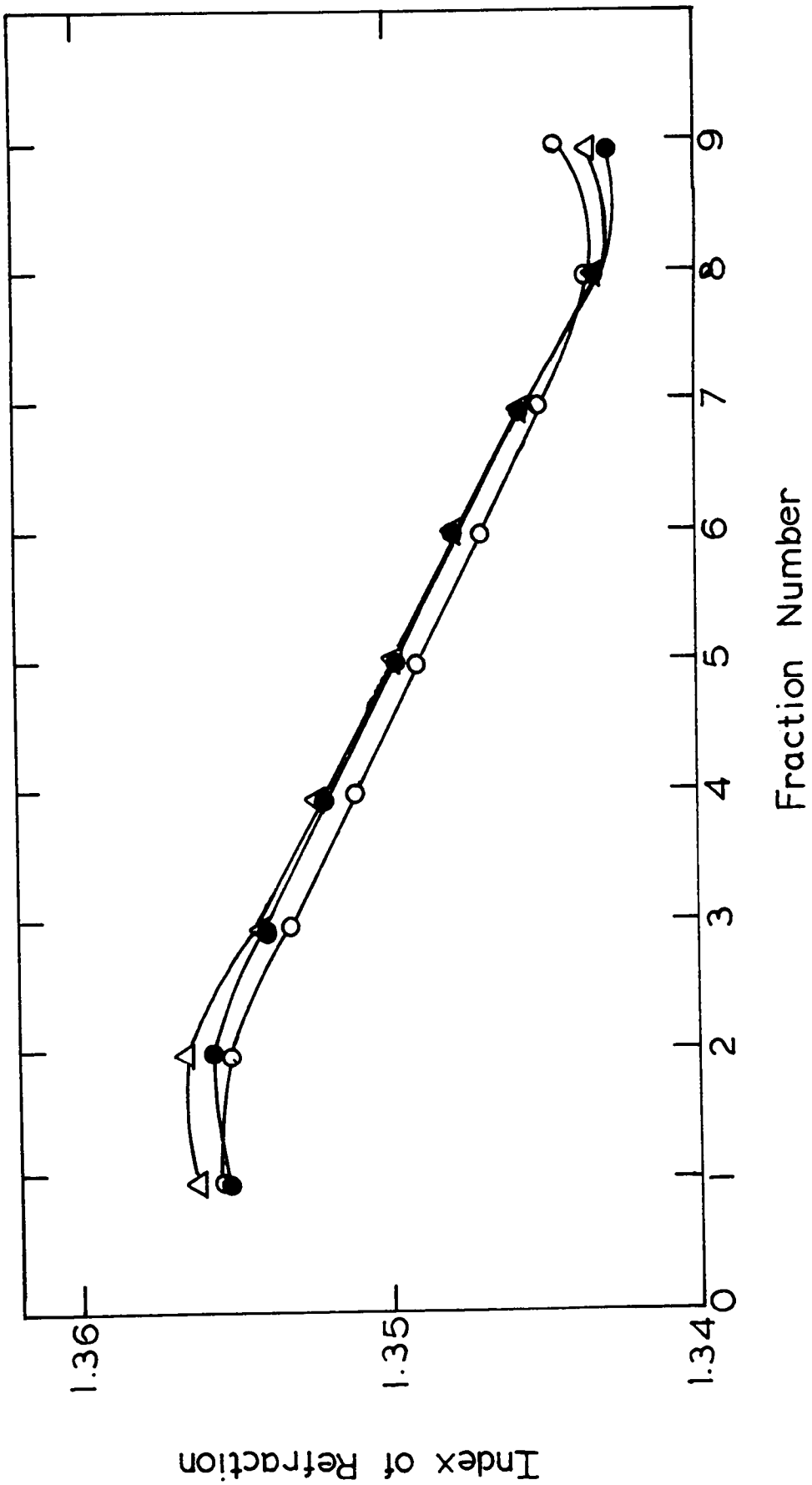


Figure 2. Analytical Ultracentrifuge Pattern of B. licheniformis Ribosomes.

Sedimentation is from left to right. Sedimentation coefficients of the major components (in Buffer A) are 28, 47, 63 and 113S. The centrifugation performed at 60,000xg and the photograph was taken 24 minutes after reaching speed. The bar angle was 65°.

Figure 3. Density Gradient Pattern of B. licheniformis Ribosomes

Ribosome concentration (Absorbance at 260nm):

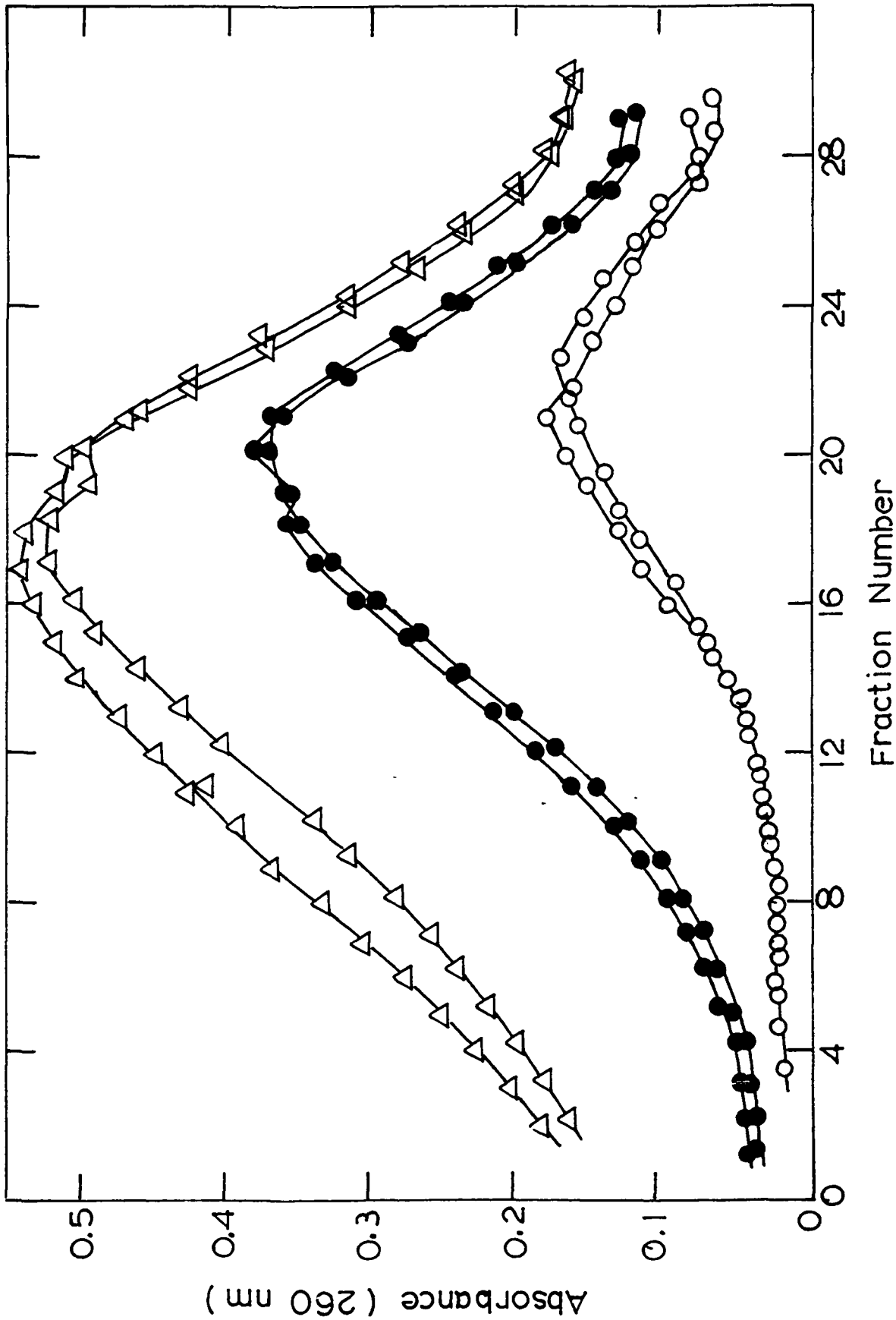
△ - 477

● - 239

○ - 95

(Symbols for each two curves)

0.1 ml of ribosome solution was applied. The density gradient tubes were centrifuged at 50,000xg for 150 minutes.



because of partial unfolding as a result of increasing hydrophilic and decreasing hydrophobic interactions.

In addition to the fact that the sucrose gradient did not lead to a good separation of ribosomal components, it appeared that such concentrated sucrose solutions were affecting the integrity of the ribosomal structures. Thus it was found that upon incubation of the ribosomes for 12 hours at $4^{o}C$ in 10% sucrose, there was an increase in 30S and 50S subunits and a decrease in 70S monomers. Hence, density gradient centrifugation did not appear to be a suitable method for fractionation of B. licheniformis ribosomes.

## Variation in Ribosome Preparation

These experiments were undertaken in the hope of so modifying the ribosome preparation in such a way that the final sample would consist entirely, or at least primarily, of 70S ribosomes. None of the modifications tried as is described below, achieved the desired result.

### Effect of Potassium Chloride Washings

Since it is known that potassium chloride washings effectively purify E. coli ribosomes (18,19), it was of interest to see whether such purification of B. licheniformis ribosomes may result in a ribosome preparation consisting of fewer components.

Accordingly, the ribosomes were subjected to washings (once after the first high speed centrifugation as described in (15,16) ) with two types of buffer containing two concentrations of potassium chloride as shown in Table I. The ribosomes were then analyzed by means of the analytical ultracentrifuge and the relative amounts of the various components were determined by measuring the areas under the peaks.

It can be seen that washing with 0.1M potassium chloride did not alter the distribution of ribosomes. On the other hand, washing with 2M potassium chloride led to a dissociation (hence a decrease) of the 70S and 100S ribosomes with a concomitant increase of 30S and 50S ribosomes. This was especially pronounced in the more dilute buffer. These results can be understood in terms of the ratio of monovalent to divalent ions, i.e. the R value (11). With the 0.1M potassium chloride wash, the R value was still below 10 and hence both 70S and 100S are expected to be stable (11). With the 2M potassium chloride wash, the R value was 200 and 400 for the 0.01M and 0.005M buffer, respectively. At such high R values, ribosomes unfold and dissociate and this could be expected to be more pronounced in the dilute buffer because of increased competition for magnesium ions.

Table I. Effect of Potassium Chloride Washing on Ribosome Patterns.

| Buffer Composition (M) | | | Relative Amounts of Ribosomes (%) | | | |
|---|---|---|---|---|---|---|
| Tris | $Mg^{++}$ | KCl | 30S | 50S | 70S | 100S |
| 0.005 | 0.005 | - | - | 15 | 35 | 50 |
| 0.005 | 0.005 | 2.0 | 5 | 62 | 33 | - |
| 0.010 | 0.010 | - | - | 9 | 34 | 56 |
| 0.010 | 0.010 | 0.1 | - | 9 | 38 | 53 |
| 0.010 | 0.010 | 2.0 | 2 | 23 | 60 | 15 |

Effect of Dialysis

The possibility of using dialysis to alter the ribosome distribution was investigated and the results are shown in Table II. The ribosomes were prepared in 0.01M (magnesium acetate, 0.01M Tris, pH 7.4) buffer (15,16) and then dialyzed in 0.005M (magnesium acetate, 0.005M Tris, pH 7.4) buffer for 24 hours at 4°C. The ribosomes so obtained were compared with ribosome prepared in 0.005M (magnesium acetate, 0.005M Tris, pH 7.4) buffer in the analytical ultracentrifuge. It can be seen that dialysis of the final ribosome preparation led to some dissociation of 100S dimers and a concomitant increase of 50S and 30S subunits.

Effect of Magnesium Ion Concentration

The results of varying the magnesium ion concentrations are shown in Table III. The buffers indicated in the table were used to dissolve the ribosomal pellets obtained from high speed centrifugation (15,16) and the final dialysis step was omitted. A decrease in the magnesium ion concentration led to a decrease of 100S and 70S ribosomes and to an increase of 50S and 30S subunits.

Effect of Tris Concentration

The results of varying the tris concentrations are

Table II. Effect of Dialysis on Ribosome Patterns.

| | Buffer Composition (M) | | Relative Amounts of Ribosomes (%) | | | |
|---|---|---|---|---|---|---|
| | Tris | $Mg^{++}$ | 30S | 50S | 70S | 100S |
| a)* | 0.005 | 0.005 for isolation | - | 13 | 40 | 48 |
| b) | 0.010 | 0.010 for isolation, | 3 | 20 | 40 | 37 |
| | 0.005 | 0.005 for dialysis | | | | |

*Average of two experiments

Table III. Effect of Magnesium Ion Concentration on Ribosome Patterns.*

| Buffer Composition (M) | Relative Amounts of Ribosomes (%) | | | |
|---|---|---|---|---|
| $Mg^{++}$ | 30S | 50S | 70S | 100S |
| 0.0010 | 16 | 69 | 15 | - |
| 0.0020 | 11 | 44 | 28 | 17 |
| 0.0025 | 7 | 35 | 33 | 25 |
| 0.0030 | 4 | 28 | 39 | 29 |
| 0.0050 | - | 14 | 39 | 47 |

* 0.005M Tris, pH 7.4

shown in Table IV. The buffers indicated in the table were used to dissolve the ribosomal pellets obtained from the high speed centrifugation (15,16) and the final dialysis step was omitted. There was no significant change in the percentages of ribosomes as a function of tris concentration. Hence, tris ions could not replace magnesium ions in stabilizing the ribosomes.

Effect of pH

The results of varying the pH are shown in Table V. The buffers indicated in the table were used to dissolve the ribosomal pellets obtained from high speed centrifugation (15,16) and the final dialysis step was omitted. At pH 7.8, there was a decrease of 100S ribosomes accompanied by an increase of 50S subunits and the appearance of 30S subunits. There was no difference in the percent of 50S, 70S and 100S ribosomes in either pH 7.4 or pH 7.0 buffer.

Effect of Various Reagents

Buffers containing various reagents were used to dissolve the ribosomal pellets obtained from each high speed centrifugation (15,16). The final dialysis step was omitted. The results are shown in Table VI.

Spermidine led to the disintegration of the whole ribosome as well as its subunits.

Table IV. Effect of Tris Concentration on Ribosome Patterns.*

| Buffer Composition (M) | Relative Amounts of Ribosomes (%) | | | |
|---|---|---|---|---|
| Tris | 30S | 50S | 70S | 100S |
| 0.005 | 16 | 69 | 15 | - |
| 0.010 | 12 | 71 | 17 | - |

* 0.001M magnesium acetate, pH 7.4

Table V. Effect of pH on Ribosome Patterns.*

| pH | Relative Amounts of Ribosomes (%) | | | |
|---|---|---|---|---|
| | 30S | 50S | 70S | 100S |
| 7.0 | - | 15 | 38 | 48 |
| 7.4 | - | 14 | 39 | 47 |
| 7.8 | 3 | 22 | 41 | 34 |

*0.005M Tris and 0.005M magnesium acetate

Table VI. Effect of Various Reagents on Ribosome Patterns*

| Reagent Added (M) | | Relative Amounts of Ribosomes (%) | | | |
|---|---|---|---|---|---|
| | | 30S | 50S | 70S | 100S |
| None | | 11 | 44 | 28 | 17 |
| Potassium chloride | 0.050 | 10 | 65 | 14 | 11 |
| Calcium chloride | 0.010 | - | - | 37 | 63 |
| Spermidine** | 0.012 | - | - | - | - |
| 2-Mercapto-ethanol | 0.010 | 10 | 45 | 35 | 11 |
| Potassium chloride and 2-Mercapto-ethanol | 0.050<br>0.010 | 10 | 65 | 14 | 11 |

* 0.005M Tris and 0.002M magnesium acetate, pH 7.4

** No sedimentable particles visible by either Schlieren or U. V. optics.

Mercaptoethanol led to a slight increase of 70S monomers probably by preventing the oxidation of critical sulfhydryl-groups.

Potassium chloride led to a decrease of 70S ribosomes probably by displacing some essential magnesium ions from the ribosomes. The presence of mercaptoethanol could not reverse this so that the bonds disrupted apparently did not involve sulfhydryl-groups.

Calcium chloride led to a large increase of 100S and a slight increase of 70S ribosomes. No 50S or 30S subunits were visible, indicating that all of them had either formed 70S or 100S. Calcium ions, being divalent and of about the same size as magnesium ions, probably bind at the same sites as magnesium ions. Hence, in the presence of added calcium ions, the results would be expected to be similar to those obtained in the presence of high magnesium ions resulting in only 70S and 100S ribosomes. It can be seen from Table VI that this was the case.

## Effect of Centrifugation

After the ribosomes were sedimented by the first high speed centrifugation (15,16), the pellets were resuspended and various cycles of centrifugation were performed in order to purify the ribosomes. The final dialysis was omitted.

The results of centrifugation are shown in Table VII. As can be seen that there was an increase in 70S monomers accompanied by a decrease of 100S ribosomes.

## Gel Filtration Chromotography

For these experiments, the ribosomes were isolated by a modified procedure (see Methods) resulting in a preparation consisting primarily of 70S and 100S ribosomes. The analytical ultracentrifuge pattern is shown in Figure 4. The gel filtration pattern of the same ribosome preparation is shown in Figure 5. Fractions from each peak were examined by analytical ultracentrifugation (using U.V. optics). In this manner, each peak was shown to consist of only one component; the first peak consisted of only 70S ribosomes, and the second peak consisted of only 100S ribosomes. The relative amounts of the 70S and 100S ribosomes corresponded to those in the unfractionated preparation.

The 70S peak was collected and concentrated by centrifugation at 105,000xg for 65 minutes. The pellet obtained was dissolved in a small amount of buffer and examined in the analytical ultracentrifuge. The 100S peak was concentrated and analyzed in the same manner. The results are shown in Table VIII. The 70S fraction reveals two peaks after concentration, a major 70S peak and a minor 100S peak. The 100S fraction likewise

Table VII. Effect of Centrifugation on Ribosome Pattern*

| Ribosome Isolation Procedure** | Relative Amounts of Ribosomes (%) | | | |
|---|---|---|---|---|
| | 30S | 50S | 70S | 100S |
| Two cycles of low- and high-speed centrifugation*** | - | 13 | 39 | 49 |
| Two cycles of low-, medium- and high-speed centrifugation**** | - | 12 | 58 | 30 |

* 0.005M Tris and 0.005M magnesium acetate, pH 7.4.

** These procedures were performed after the first high-speed centrifugation (15,16).

*** Twenty minutes at 12,000xg followed by 65 minutes at 105,000xg and resuspension of the pellet (used in reference 15,16).

**** Twenty minutes at 12,000xg; 30 minutes at 10,000xg (pellet discarded) followed by 65 minutes at 105,000xg and resuspension of the pellet.

Figure 4. Analytical Ultracentrifuge Pattern of *B. licheniformis* Ribosome

Sedimentation is from left to right. Sedimentation coefficients of the major components (in complete buffer B) are 66 and 93S. The centrifugation was performed at 60,000xg and the picture was taken at 15 minutes after reaching speed. The bar angle used was $60^{o}$.

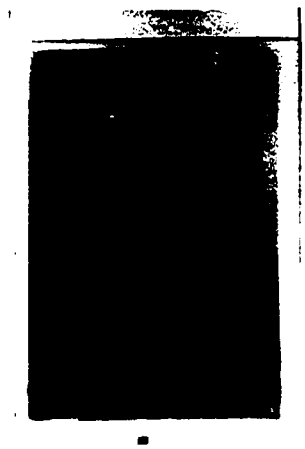


Figure 5. Gel Filtration Pattern of *B. licheniformis* Ribosomes

Buffer B was used and 0.8 ml of ribosome solution (absorbance of 282 at 260 nm) was applied. The flow rate was 39 ml/hr. The absorbance plotted in Figure 5 is the absorbance of the column eluate (undiluted).

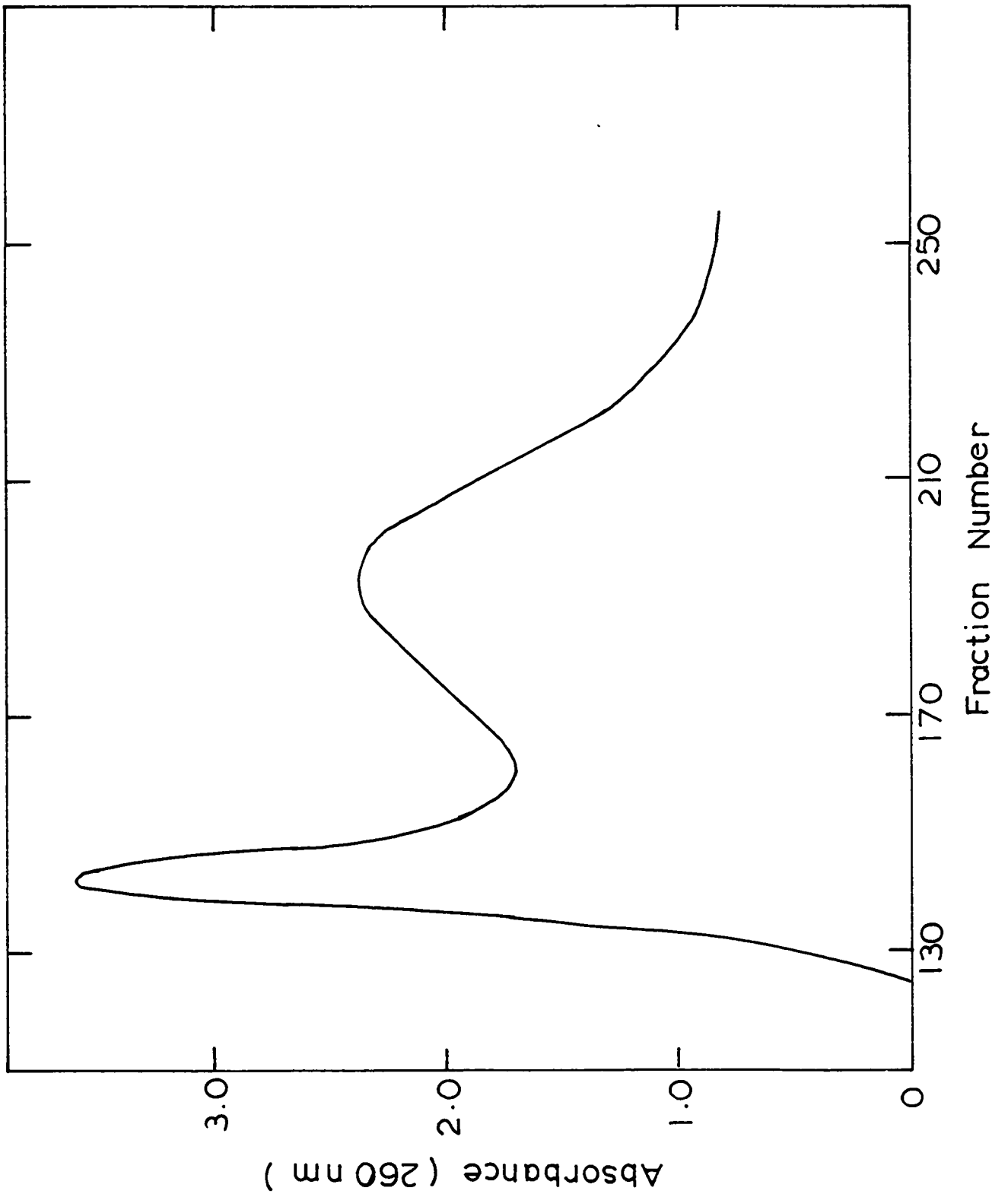


Table VIII. Concentration by Centrifugation of Ribosome Fractions from Gel Filtration

| Conditions | Component ($S_{20}$) | Relative Amount (Area*) |
|---|---|---|
| Unfractionated ribosomes | 66 | 1806 |
| | 93 | 1077 |
| Concentrated 70S fraction | 65 | 1135 |
| | 96 | 735 |
| Concentrated 100S fraction | 66 | 387 |
| | 96 | 593 |

* Area ($cm^2$) under the peaks obtained in the analytical ultracentifuge and corrected for optical factors and radial dilution.

indicates two peaks, a major 100S peak and a minor 70S peak. The amounts of the minor peaks were such that they would have been detected in the original 70S or 100S peak had they been present. Thus the minor peak in each case must have arisen from the major one in the course of concentration. Attempts to concentrate the eluted fraction using Sephadex G-25 were unsuccessful.

In order to assess the resolving power of the method, it was desirable to have a sample containing 30, 50, 70 and 100S ribosomes. This was achieved by dialyzing the final ribosome preparation versus buffer consisting of 0.005M Tris and 0.002M magnesium acetate at pH 7.4. The same buffer was used for gel filtration. The analytical ultracentrifuge pattern of the ribosomes prepared in this fashion is shown in Figure 6 and their gel filtration pattern is shown in Figure 7. Fractions from the peak consisted of well resolved 100S ribosomes. The second peak consisted of partially separated 30, 50 and 70S ribosomes. The relative amounts of these ribosomes varied with the fraction analyzed (more 30S at the leading edge of the peak, 50S at the middle and 70S at the trailing edge of the peak) and are shown in Table IX.

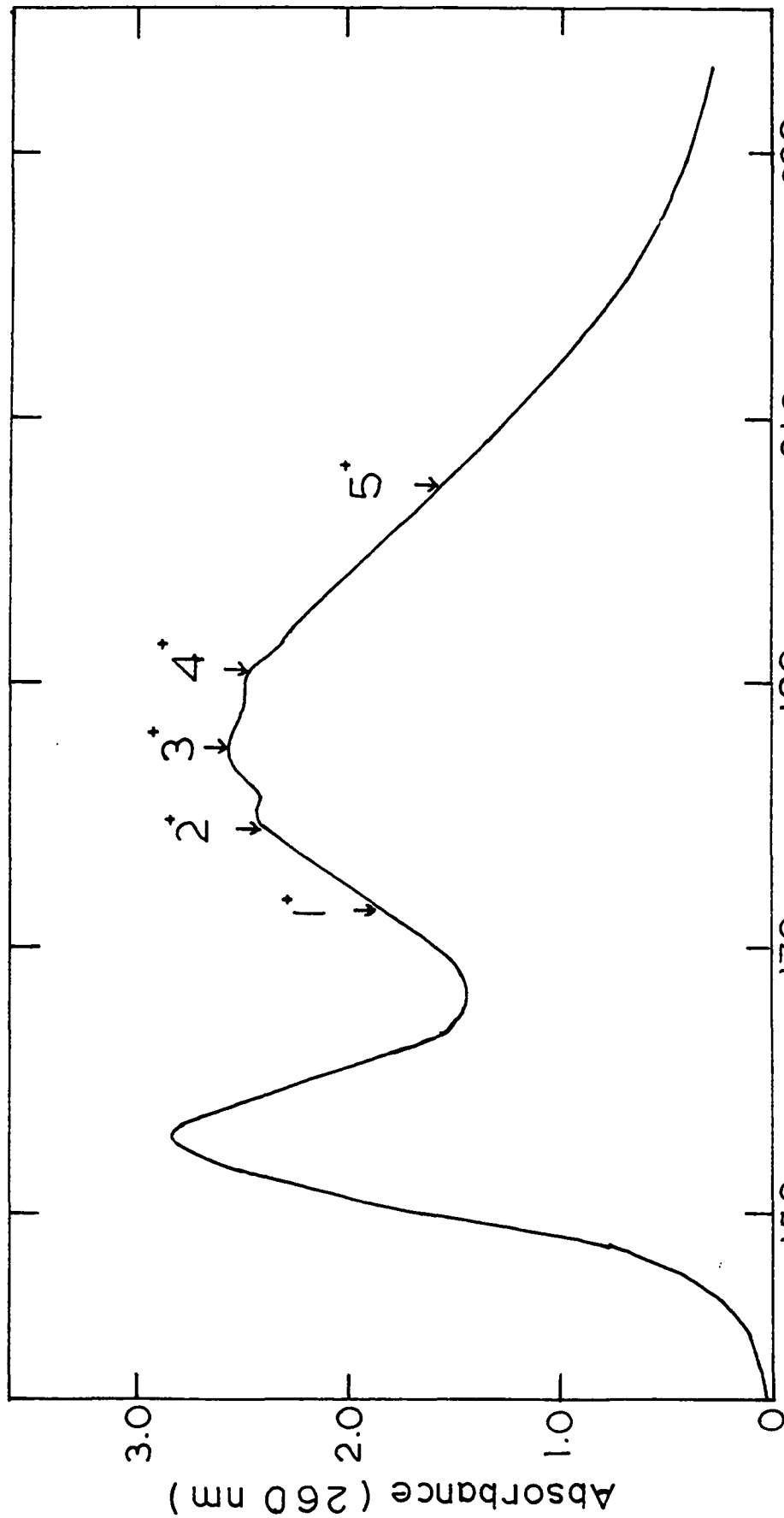
The recovery of applied ribosomes in both cases (Figs. 5 and 7) was about 100% base on absorbance measurements at 260 nm. The ribosomes were eluted slightly

Figure 6. Analytical Ultracentrifuge Pattern of *B. licheniformis* Ribosomes

Sedimentation is from left to right. Sedimentation coefficients of the major components (in Buffer C) are 30,49,63 and 102S. The centrifugation was performed at 60,000xg and the photograph was taken at 25 minutes after reaching speed. The bar angle used was 60°.

Figure 7. Gel Filtration Pattern of *B. licheniformis* Ribosomes

Buffer C was used and 0.5 ml of ribosome solution was applied. The flow rate was 30 ml/hr. The absorbance plotted in Figure 7 is the absorbance of the column eluate (undiluted).




+ The composition of samples 1,2,3,4,5 is shown in Table IX.

Table IX. Composition of Gel Filtration Fractions of *B. licheniformis* Ribosomes

| Sample Number* | Relative Amounts of Ribosomes (%) | | |
|---|---|---|---|
| | 30S | 50S | 70S |
| 1 | 7 | 32 | 60 |
| 2 | 7 | 36 | 57 |
| 3 | 9 | 41 | 51 |
| 4 | 14 | 47 | 39 |
| 5 | 22 | 43 | 35 |

* Sample number are indicated on the curve in Figure 7.

after the void volume which was determined with Blue Dextran 2000 (Pharmacia Fine Chemicals).

## SUMMARY

Three approaches were used to fractionate the ribosomes isolated from the mesophilic bacterium, Bacillus licheniformis, with the aim of obtaining a pure 70S ribosome fraction.

One approach involved sucrose density gradient centrifugation. Under these conditions, the 70S ribosomes were not well separated from the other ribosomes and were also unstable in sucrose solutions.

A second approach involved variations in the ribosome preparation. These included variations of tris and magnesium ion concentrations, pH, centrifugation conditions, ribosome washings with potassium chloride, and the addition of various reagents to the ribosomal solution. None of these variations produced a preparation composed of only 70S ribosomes.

The third approach involved gel filtration. By the use of this method it was possible to isolate a pure 70S ribosome fraction from an improved ribosome preparation, which consisted of 70S and 100S ribosomes. Ribosome preparations consisting of 30, 50, 70 and 100S were partially separated by this technique; the 100S ribosomes could be obtained as a pure component while the other three types of ribosomes were only partially separated.

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