8-1967

Stability and Physical Parameters of Ribosomes from Mesophilic and Thermophilic Bacteria

Cynthia R. Y. Yang

STABILITY AND PHYSICAL PARAMETERS OF RIBOSOMES FROM MESOPHILIC AND THERMOPHILIC BACTERIA

by

Cynthia R. Y. Yang

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

Western Michigan University
Kalamazoo, Michigan
August, 1967

ACKNOWLEDGEMENTS

The author wishes to express her sincere appreciation to Dr. Jochanan Stenesh for his patience and inspiring guidance which made the completion of this research possible.

Further acknowledgement is given to the American Cancer Society and to the National Institute of Allergy and Infectious Diseases for their financial support.

TABLE OF CONTENTS

TABLE OF CONTENTS

Continued

INTRODUCTION

A majority of organisms carry on their normal physiological processes in a moderate range of temperatures (about 20° to 45° C). These organisms are termed mesophiles. Certain organisms, however, grow at much higher temperatures (about 55° to 80° C). These organisms are called thermophiles.

Three main theories have been advanced to explain the phenomenon of thermophily. One theory (1,2) considers thermophily to be a special type of metabolic state, involving high rates of breakdown and synthesis of cell constituents. A second interpretation relates thermal stability to the protective action of lipids and attempts to correlate heat stability with the melting point of the cell lipids (3). A third theory ascribes thermophily to physical and chemical differences of macromolecules as compared to similar macromolecules from mesophiles.

Most of the evidence accumulated so far supports the latter theory. Such evidence has come largely from studies of proteins and nucleic acids. Koffler (4) showed that cytoplasmic proteins from thermophilic bacteria are more heat stable than those from mesophilic bacteria. Stenesh and Koffler (5) demonstrated a striking difference in heat stability for flagella from those two types of bacteria. Campbell (6,7) showed that crystalline α-amylase isolated from cultures of Bacillus coagulans grown at 55°C was more heat stable than the same enzyme isolated from

cultures grown at 37°C. Recently, Stenesh and Holazo (8) showed that the ribosomal RNA from thermophilic strains of Bacillus was more heat stable than the ribosomal RNA from mesophilic strains of Bacillus. The RNA from the thermophiles melted out at a higher temperature and had a higher guanine plus cytosine content than that from the mesophiles. The DNA (9) and the soluble RNA (10,11) from thermophilic strains of Bacillus showed no unusual heat stability when compared to similar components of Escherichia coli. On the other hand, ribosomes from thermophilic strains of Bacillus were more heat stable than those of Escherichia coli (11,12). However, such comparisons of thermophilic strains of Bacillus with mesophilic strains of E. coli do not exclude the possibility of intergeneric differences. For this reason we have been studying in this laboratory mesophilic and thermophilic strains from one genus, namely Bacillus. The present work deals with studies of the stability and the physical parameters of ribosomes isolated from these strains.

Ribosomes are macromolecular particles of the cytoplasm of all kinds of cells. They are attached to the endoplasmic reticulum of cells of various animals and plants, while they occur free in bacterial extracts. They are also called ribonucleoprotein particles because they contain 40-65% RNA and 60-35% protein, with little or no lipid. These particles are fairly constant from cell to cell in their size, shape, and chemical composition. They are about 100-250 Å in diameter and are roughly spherical.

Ribosomes can dissociate into subunits. They can also associate into dimers, trimers, etc., and aggregate into larger, polydisperse masses. The degree of dissociation or association of the ribosomes is a function of the magnesium ion concentration. One fascinating aspect of ribosomes is the fact that the individual particles have no protein synthesizing ability, but only clusters of ribosomes are active in protein synthesis. These clusters have been termed polysomes, ergosomes, polyribosomes, or heavy ribosomes. Of these, the most frequently encountered enzyme is a ribonuclease. The function of this enzyme is not known.

Isolation of ribosomes can be readily accomplished by fractional centrifugation of a homogenate of bacterial cells and the procedures will be described in detail later.

The stability of the ribosomes was investigated by following the ribosome breakdown as a result of the digestion by the associated ribonuclease. This enzyme can be activated by chelating magnesium ions which normally inhibit the enzyme. Two types of experiments were carried out under these conditions: (1) the effect of temperature on ribosome stability, (2) the effect of various activators and inhibitors on the ribosomal ribonuclease.

The studies of the physical parameters of the ribosomes involved determinations of sedimentation coefficients, diffusion coefficients, and molecular weights.

MATERIALS AND METHODS

Organisms and Growth Conditions

Three mesophilic and three thermophilic strains of the genus Bacillus were used. The former included B. licheniformis (NRS 243), B. pumilus (NRS 236), and B. sp. (X-1). The thermophiles were strains of B. stearothermophilus (FJW,10,2184).

The bacterial cultures were grown in slants and large petri dishes on a medium containing 2% Bacto agar (Difco), 1% Trypticase (BBL), and 0.2% Yeast extract (Difco). Slants which contained in addition to the above 10 p.p.m. of manganese were used for growing the stock cultures. Slants were grown to the logarithmic phase and the growth was suspended in about 6 ml of sterile water. Two ml of this suspension were used to inoculate one petri dish. The petri dishes were then incubated and the cells harvested in the logarithmic phase (approximately 6 hr at 37°C for the mesophiles and 5 hr at 55°-60°C for the thermophiles, for both slants and petri dishes).

Reagents

Buffer A consisted of 0.01 M tris(hydroxymethyl)aminomethane (Sigma) and 0.01 M magnesium acetate (Baker). Buffer B consisted of 0.005 M tris and 0.005 M magnesium acetate. Both buffers were adjusted to pH 7.4 with 1 N hydrochloric acid. The alumina used was a chromatographic grade preparation (Merck 71707). The

deoxyribonuclease (Worthington Biochemical Corp., electrophoretically purified) was stored frozen in buffer A at a concentration of 100 ug/ml.

Other reagents used were the following: 0.05 M $Na_2HAsO_4 \cdot 7H_2O$ (Baker), 0.05 M $Na_2HPO_4 \cdot 7H_2O$ (Baker), 0.05 M ethylene diamine tetraacetic acid (EDTA; Matheson; adjusted to pH 7.4 with 2.5 N NaOH), and 5% perchloric acid (v/v), prepared from 70% reagent perchloric acid (Mallinckrodt).

Apparatus

All spectrophotometric measurements were made in a Zeiss model PMQ-II spectrophotometer.

A Sorvall model RC-2 refrigerated centrifuge was used for low speed centrifugations (15,000 r.p.m. and below), and a Spinco model L preparative ultracentrifuge was used for high speed centrifugations (above 15,000 r.p.m.). All analytical ultracentrifugation studies were made in a Spinco model E analytical ultracentrifuge, equipped with rotor temperature indicator control and with schlieren and ultraviolet optics. All runs were made between 4° and 10°C. Measurements of the photographic plates and films were made with a Nikon (model 6C) microcomparator and a Joyce-Loebl (chromoscan) densitometer.

All area measurements were made with a Gelman (model 39231) planimeter.

Density and viscosity measurements were made with a Weld pycnometer and an Ostwald viscometer, respectively.

Isolation of the Ribosomes

The procedure is essentially as described previously (13). Buffer A was used throughout the isolation. Cells in the logarithmic phase of growth were harvested with buffer and were collected by centrifugation for 25 minutes at 12,000 x g in a Sorvall (model RC-2) with the GSA rotor. The cells were then ground with 3 parts by weight of alumina for 3-5 minutes and extracted with 10 volumes of buffer. Deoxyribonuclease was added to a final concentration of 1 ug/ml. This mexture was kept at 20°C for 30 minutes. Cell debris and alumina were then removed by centrifugation for 20 minutes at 12,000 x g in the Sorvall, the supernatant was collected and was centrifuged in a Spinco (model L) ultracentrifuge for 65 minutes at 105,000 x g or for 95 minutes at 78,500 x g to sediment the ribosomes. Two cycles of low- and high-speed centrifugation (20 minutes at 12,000 x g followed by 65 minutes at 105,000 x g and gentle resuspension of the pellets with a policeman) were performed to purify the ribosomes. Finally, a low speed centrifugation (20 minutes at 12,000 x g) was performed to remove the aggregates and the supernatant provided the final ribosomal preparation for the enzyme studies.

For the studies dealing with the physical parameter of the ribosomes, some modifications were made in the purification of the ribosomes. After each high speed centrifugation, the ribosomal pellets were suspended by means of gentle homogenization using a teflon homogenizer. Buffer A was used throughout and the

concentration of the ribosomes was kept as high as possible. The final solution from the low speed centrifugation was dialyzed against 400 volumes of buffer B for 24 hours, with changes of buffer solution every 6 hours. After dialysis, the solution was centrifuged at 27,000 x g in the Sorvall for 5 minutes. The residue was discarded and the supernatant thus obtained was the final ribosomal preparation.

Measurements of the Effect of Temperature on Ribosome Stability

The incubation mixtures were set up in test tubes. The basic incubation mixture consisted of 1.0 ml of ribosomal solution (absorbance of 5 at 260 mu), 10 umoles of tris (pH 7.4), and 10 umoles of magnesium acetate. For these experiments each tube contained in addition to the basic incubation mixture 30 umoles of EDTA (pH 7.4), in a total volume of 2.5 ml. The tubes were tightly covered with parafilm and were incubated for one hour at 55°, 60°, 65°, 70°, and 75°C, respectively. At the end of one hour, 2.5 ml of 5% (v/v) perchloric acid was added to each tube to stop the reaction. The tubes were kept in ice for half an hour. The solution was then centrifuged at 12,000 x g for 10 minutes in the Sorvall (SS 34 rotor). The supernatant fluid was collected and its optical density (absorbance) was measured at 260 mu versus the blank which contained all the components except that 1.0 ml of buffer A was used instead of the ribosomal solution. Since RNA and RNA breakdown products absorb maximally at 260 mu, optical

density measurements at this wavelength provide a convenient way of following the reaction.

Studies Involving Activators and Inhibitors of the Ribosomal Ribonuclease

Four sets of reaction mixtures were set up in test tubes. Each tube contained a total of 2.5 ml of solution. One of the sets contained a basic incubation mixture only, that is, 1.0 ml of ribosomal solution (absorbance of 5 at 260 mu), 10 umoles of tris (pH 7.4), and 10 umoles of magnesium acetate. The other sets contained in addition to the above, either 30 umoles of EDTA (pH 7.4), or 30 umoles of EDTA (pH 7.4) and 25 umoles of Na_2HAsO_4, or 30 umoles of EDTA (pH 7.4) and 25 umoles of Na_2HPO_4. The tubes were tightly covered and were incubated at 45°C for various lengths of time. At various time intervals, one tube from each set was removed from the water bath and the reaction stopped by the addition of 2.5 ml (v/v) of perchloric acid. The tubes were kept in ice for half an hour and the solution was then centrifuged as described in the previous section. The supernatant fluid was collected and its absorbance at 260 mu was read versus zero-time controls which contained the same components as the incubation mixture except that perchloric acid was added immediately after the addition of the ribosomal solution.

Determination of the Sedimentation Coefficients of the Ribosomes

The sedimentation coefficients were obtained from sedimentation velocity experiments using the Spinco (model E)

analytical ultracentrifuge. The AND rotor was used for solutions at higher concentrations (OD_{260}-optical density at 260 mu- of 50 or above for schlieren optics, OD_{260} of 0.5 or above for ultraviolet optics), while the ANE rotor was used for the more dilute solutions. The double sector cell was used with the AND rotor so that a base line could be obtained. Most runs were made at 29,500 r.p.m., but some were performed at 25,980 r.p.m. Pictures were taken automatically every four minutes. The schlieren plates were developed for four minutes with Kodak D-19 developer, washed with 3.4% acetic acid and fixed for half an hour with Kodak Fixer. The plates were then thoroughly washed with water and air dried. Measurements of the plates were done with a Nikon microcomparator. Ultraviolet films were developed with Kodak D-11 developer for eight minutes, washed with 3.4% acetic acid, fixed with Kodak Fixer and washed and dried like the schlieren plates. Measurements of the films were made with a Joyce-Loebl densitometer. The distances between boundaries and the center of rotation were calculated and were expressed in centimeters. Logarithms of these distances were plotted versus time (in minutes) at which the particular picture was taken. The observed values of the sedimentation coefficients were computed from the slope since the sedimentation coefficient is defined as $s = \frac{dx/dt}{w^2x}$ where x is the distance of the boundary from the center of rotation in centimeters, t is the time of centrifugation in seconds and w is the angular velocity in radians/sec.

The observed values of the sedimentation coefficients (s_{obs}) were corrected to 20°C for the viscosity of water and were expressed as s_{20}^{w} in Svedberg units (S) as shown in the following equation:

$$s_{20}^{w} = s_{obs}\left(\frac{\eta_t}{\eta_{20}}\right)_{H_2O}\left(\frac{\eta_{solvent}}{\eta_{H_2O}}\right)_{any\ t}\left(\frac{1-\bar{v}_{20}\rho^{H_2O}_{20}}{1-\bar{v}_t\,\rho^{solvent}_{t}}\right)$$

where η is the viscosity, $\bar{v}$ is the partial specific volume of the solute (ribosomes), t is the temperature at which the centrifugation was performed, and ρ is the density. However, the last term in the equation amounts only to a correction of about 0.1% and has been neglected.

Determination of the Diffusion Coefficients of the Ribosomes

In order to obtain the diffusion coefficients, the speed of the centrifugation was lowered to 4059 r.p.m. after each sedimentation velocity experiment. At this low speed the boundaries did not migrate any further in the cell during the time interval used for diffusion studies (about 90 minutes). Instead, the boundaries spread out because of diffusion. From this spreading of the boundaries, in the absence of appreciable sedimentation, an apparent diffusion coefficient can be calculated. Temperatures were controlled and pictures were taken in the same manner as in the determination of the sedimentation coefficients. The height of each peak above the base line was measured with a Nikon micro-comparator and was converted into centimeters in the centrifugal

cell. The reciprocal of the height squared was plotted versus time in seconds. Each area underneath the peak was measured with a Gelman planimeter and was converted to square centimeters in the cell. Observed values of the apparent diffusion coefficients were then computed from the slope and the area, since the diffusion coefficient is defined as $H_m = \frac{\text{area}}{2\sqrt{Dt\pi}}$, and hence $D = \frac{(\text{area})^2 \text{slope}}{4\pi}$, where H_m is the height of the peak in centimeters, t is the time of diffusion in seconds, and D is the apparent diffusion coefficient in cm^2/sec.

The observed values of the diffusion coefficient (D_{obs}) were then corrected to give D_{20}^{W}, the diffusion coefficient at 20°C in water, according to the following equation:

$$D_{20}^{W} = D_{obs} \left(\frac{293}{273+t}\right) \left(\frac{\eta_{\text{solvent}}}{\eta_{H_2O}}\right)_{\text{any t}} \left(\frac{\eta_t}{\eta_{20}}\right)_{H_2O}$$

where η is the viscosity, and t is the temperature at which the diffusion was performed.

Determination of the Molecular Weights of the Ribosomes

The molecular weight, M, of a macromolecule can be computed from its sedimentation coefficient, s, and its diffusion coefficient, D, according to the Svedberg equation, $M = \frac{RTs}{D(1-\bar{v}\rho)}$, where R is the gas constant in erg/mole x degree, T is the absolute temperature at which both s and D are calculated, $\bar{v}$ is the partial specific volume of the solute, and ρ is the density of the solvent used.

RESULTS AND DISCUSSION

The Effect of Temperature on Ribosome Stability

All the ribosome preparations were shown previously (13) to contain a ribonuclease activity similar to the one found in association with many other ribosomes (14,15). This enzyme, in the presence of EDTA, led to a breakdown of the ribosomes as judged by the increase in acid-soluble nucleotides (13). The ribosomes from the thermophiles were shown to be somewhat more stable to such breakdown than those from the mesophiles, at both 37° and 60°C. With this in mind, the present experiment was designed in order to determine whether this difference in stability might be more pronounced at temperatures exceeding the maximal growth temperatures (about 55°C) of the mesophiles. Accordingly, the reaction was measured over a range of temperatures from 55° to 75°C. The results are given in Figure 1. It can be seen that the ribosomes from the mesophiles are, in fact, less stable at temperatures above 60°C than those from the thermophiles. This refers to both the relative breakdown as defined in Figure 1 and to the absolute extent of degradation. These data, taken in conjunction with the previous findings (13), support the theory that thermophily is based upon chemical-physical differences on the molecular level.

Figure 1. Ribosome breakdown at various temperatures. Basic incubation mixtures (see Methods) plus 30 umoles of EDTA (pH 7.4) were incubated at various temperatures for 1 hr.

(a) Mesophilic strains: (▲) B. pumilus; (□) B. sp. (X-1); (●) B. licheniformis.

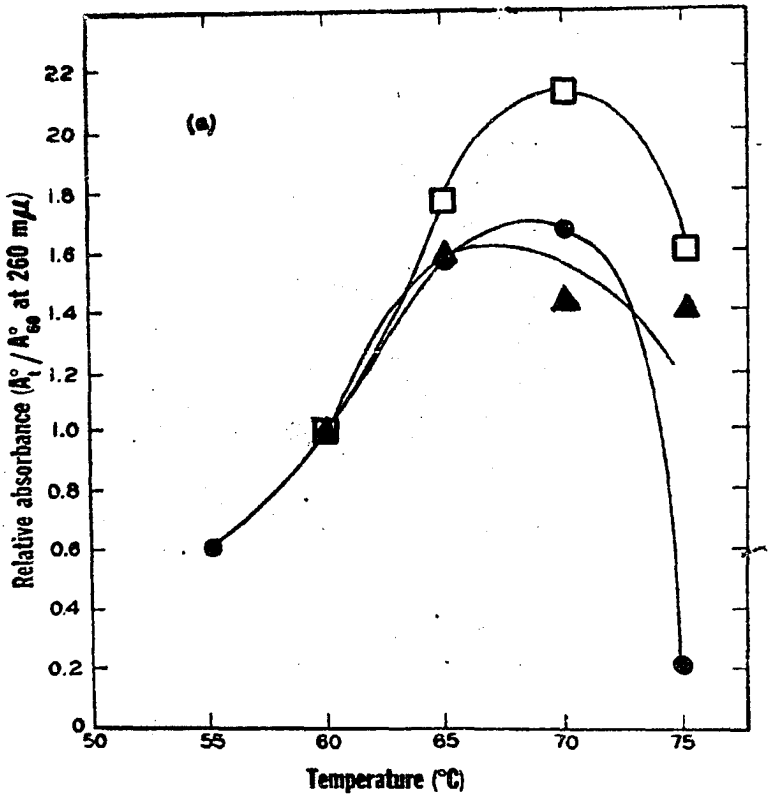
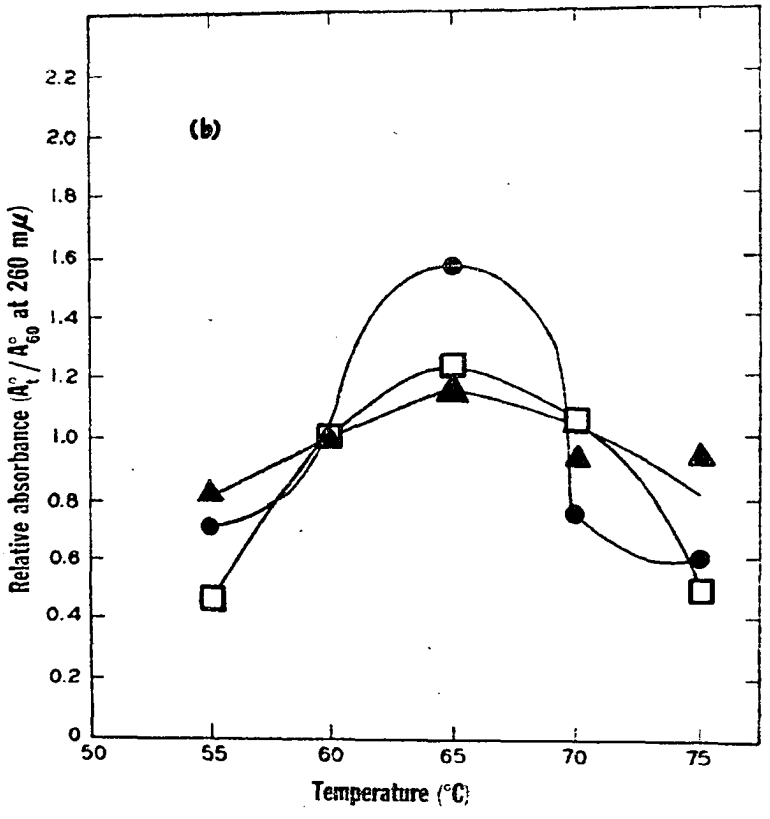


Figure 1.

(b) Thermophilic strains : (▲) B. stearothermophilus FJW; (□) B. stearothermophilus 10; (●) B. stearothermophilus 2184.



Activators and Inhibitors of the Ribosomal Ribonuclease

To evaluate properly the relative stability of the ribosomes, it was important to establish that the ribosome breakdown is due to the same enzyme in all instances. For this purpose, the ribosomes were incubated in buffer with the addition of EDTA, EDTA and phosphate, or EDTA and arsenate. The results are shown in Figure 2. It can be seen that the shapes of the curves and the magnitudes of the changes observed are very similar. The ribosome degradation in the absence of EDTA is slow, in part because of the inhibition of the enzyme by the magnesium in the buffer. This is based on the assumption that magnesium inhibits ribonucleases though admittedly there are conflicting data in the literature on the effect of magnesium on this enzyme (16,17). In common with other ribosomal ribonuclease (18,19), the inhibition by magnesium was removed by chelation of the magnesium with EDTA. The addition of either phosphate or arsenate led to a pronounced activation of the enzyme, with a resultant linear rate of ribosome breakdown. The activation by either phosphate or arsenate was essentially identical, as reported also for rat liver ribosomes(20).

These data provide good evidence that the ribosome breakdown was due to the action of a ribonuclease. This conclusion is based on the fact that other enzymes involved in RNA breakdown cannot account for the results obtained here. Phosphodiesterases are inhibited by arsenate and EDTA, but are activated by phosphate

Figure 2. Activators and inhibitors of the ribosomal ribonuclease. Basic incubation mixtures (see Methods) plus one of the following: (●) no additions; (▲) 30 umoles of EDTA (pH 7.4); (□) 30 umoles of EDTA (pH 7.4) and 25 umoles of Na_2HAsO_4; (△) 30 umoles of EDTA (pH 7.4) and 25 umoles of Na_2HPO_4. Incubation at 45°C. The relative absorbance is the absorbance of the sample divided by the absorbance (after 5 hr) of the solution containing EDTA and Na_2HPO_4.

(a) B. pumilus

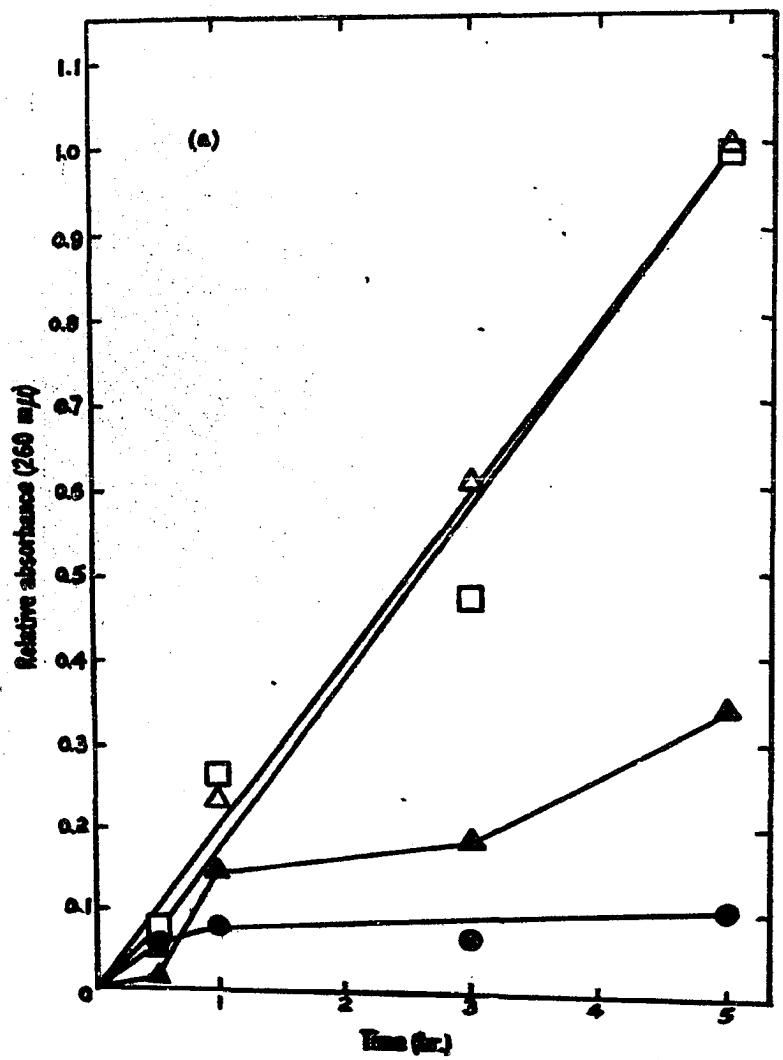


Figure 2.
(b) B. sp. (X-1)

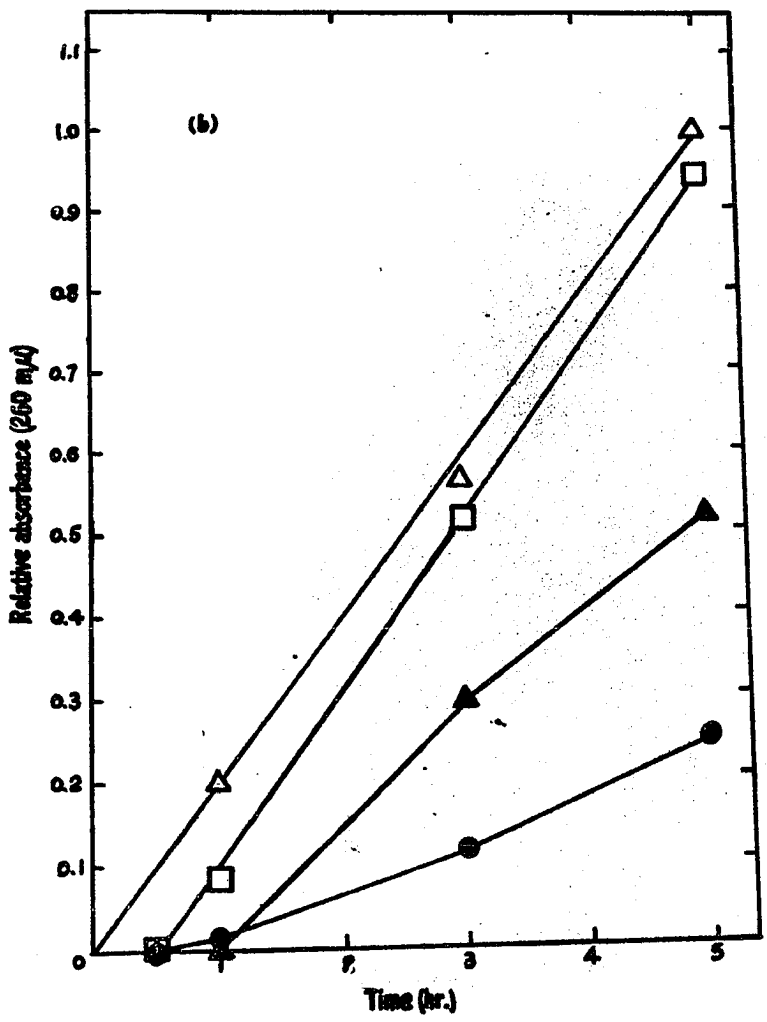


Figure 2.
(c) B. licheniformis

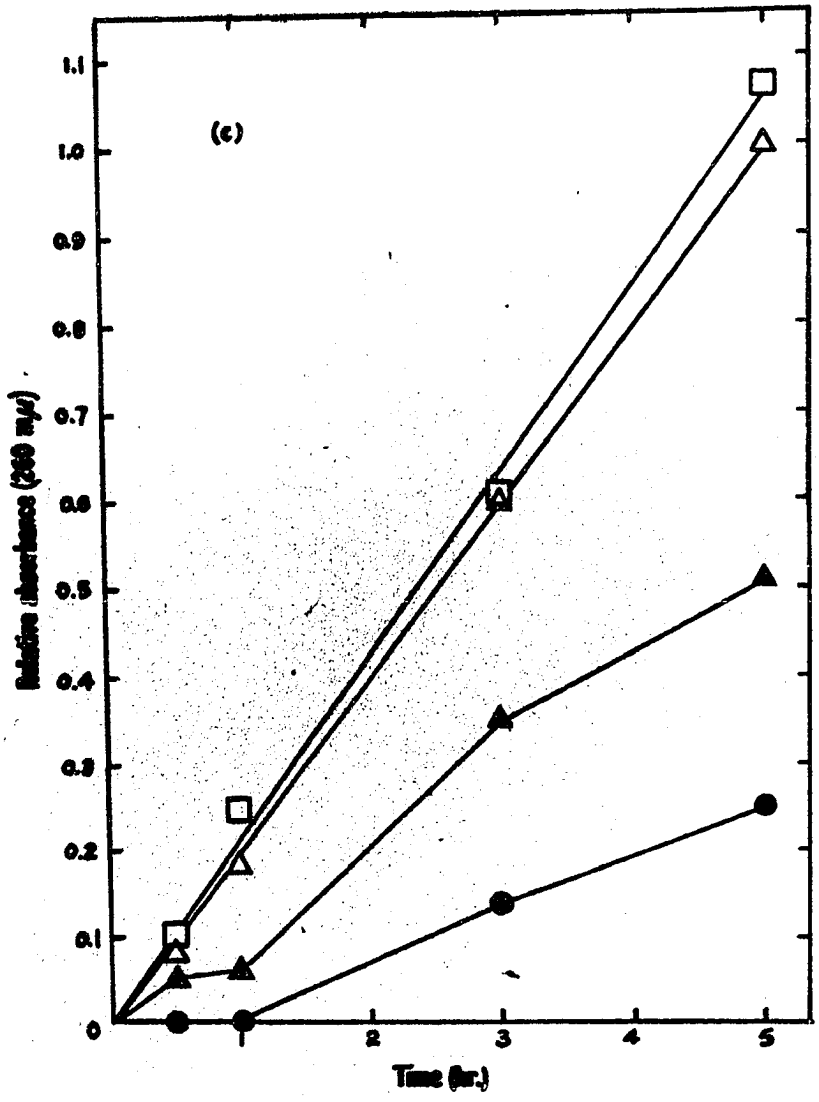


Figure 2.

(d) B. stearothermophilus FJW

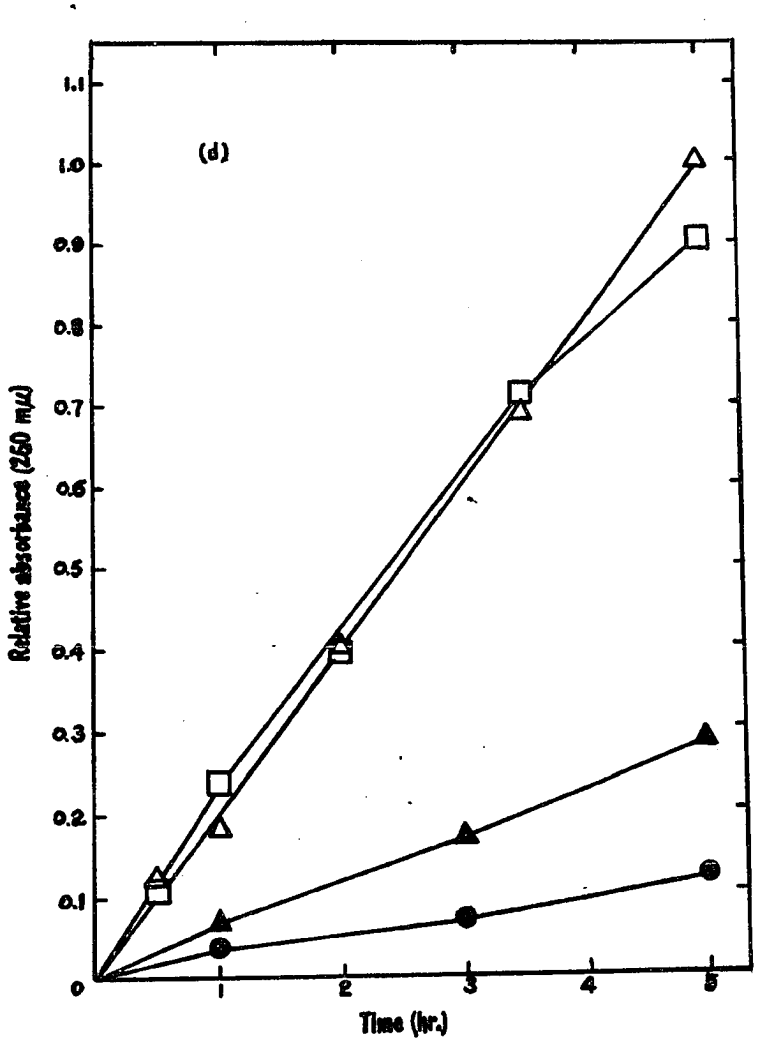


Figure 2.
(e) B. stearothermophilus 10

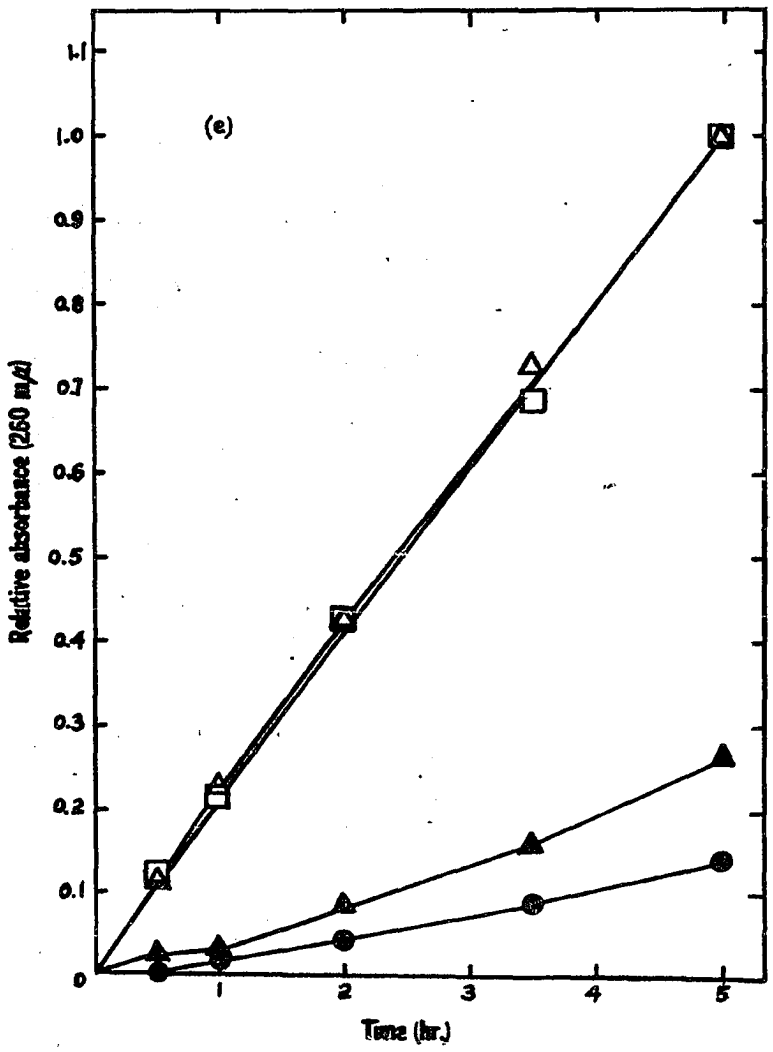
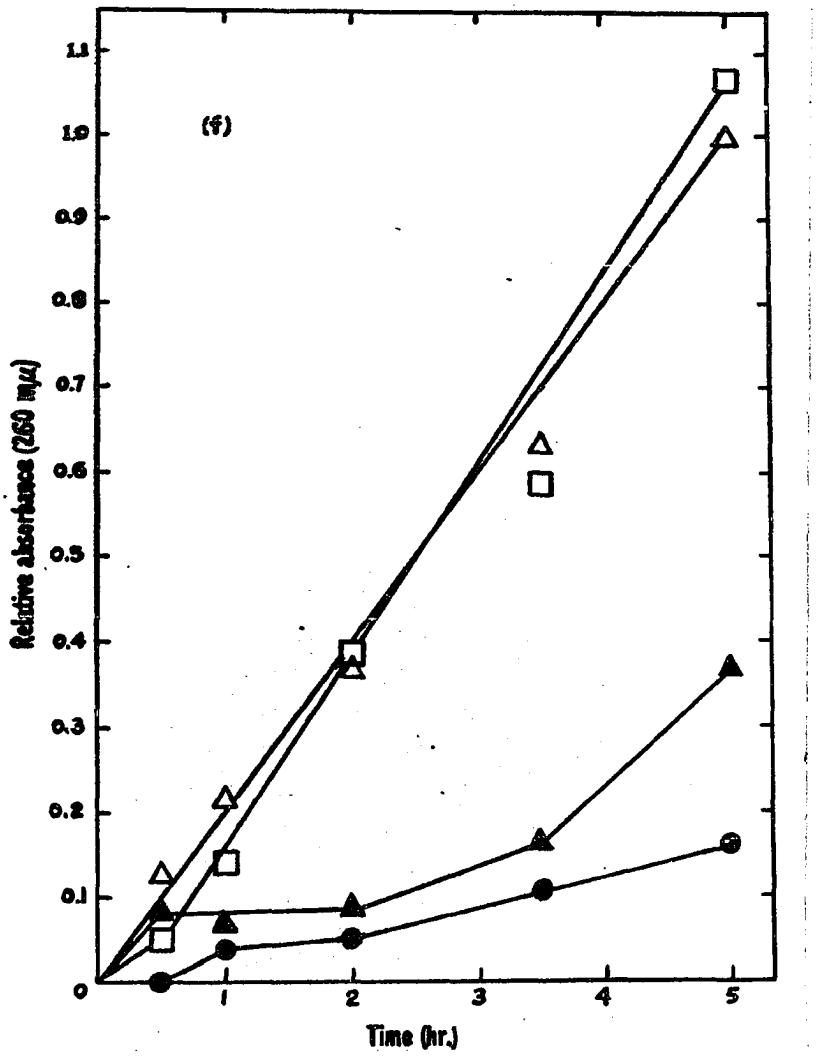


Figure 2.

(f) B. stearothermophilus 2184



(20,21); the potassium-activated phosphodiesterase found in association with E. coli ribosomes is inactivated by EDTA (21); and polynucleotide phosphorylase requires magnesium for activity (22,23). Ribonucleases, on the other hand, are inhibited by magnesium, but are activated by EDTA, arsenate, and phosphate (20).

The Effect of Magnesium Ions on the Size Distribution of the Ribosomes

In order to study the physical parameters of the ribosomes, it is desirable to obtain a ribosomal solution containing mostly, if not entirely, the fundamental ribosomal particles, that is the 70 S monomers. Since it is known that magnesium ion concentration affects the size distribution of the ribosomes, we attempted to search for a buffer solution which contained the proper concentration of magnesium and tris, so that the ribosomal solution obtained contained a bulk of 70 S monomers. We found, in fact, that tris concentration did not affect significantly the size distribution of the ribosomes as indicated by their ultracentrifuge patterns. On the other hand, the sedimenting components of the ribosomes dissociated and aggregated as a function of magnesium ion concentration in a manner that is typical of ribosomes in general. Thus, for example, the 70 S and 100 S ribosomes of B. licheniformis and of B. stearothermophilus FJW, in 0.01 or 0.005 M tris (pH 7.4), were dissociated into 30 S and 50 S particles when the magnesium ion concentration was lowered from 0.005 to 0.001 M. These particles reassociated when the magnesium ion

concentration was raised to 0.005 M. Some of these untracentrifuge patterns are shown in Figure 3.

Although the size distribution of the ribosomes was changed by varying the magnesium ion concentration (Fig. 3), we were not able to obtain a solution which contained only, or mostly, the 70 S particles. These experiments were performed with the ribosomes from a mesophile (B. licheniformis) and a thermophile (B. stearothermophilus FJW). Homogenization of the ribosomal pellets during the isolation decreased the time required for the preparation of the ribosomes but did not alter the distribution patterns of the ribosomes. This pattern was also not changed by varying the last centrifugation step in the isolation of the ribosomes (see Methods).

From these experiments, it became apparent that other techniques would be required to obtain preparations consisting only of 70 S monomers. We decided, therefore, to perform the physical measurements on the preparations at hand, using the best set of conditions, so that the 70 S monomers would be the predominant, or at least a major, ribosomal particle. This set of conditions turned out to be the isolation of the ribosomes in buffer A and dialysis of the final preparation versus buffer B (see Methods).

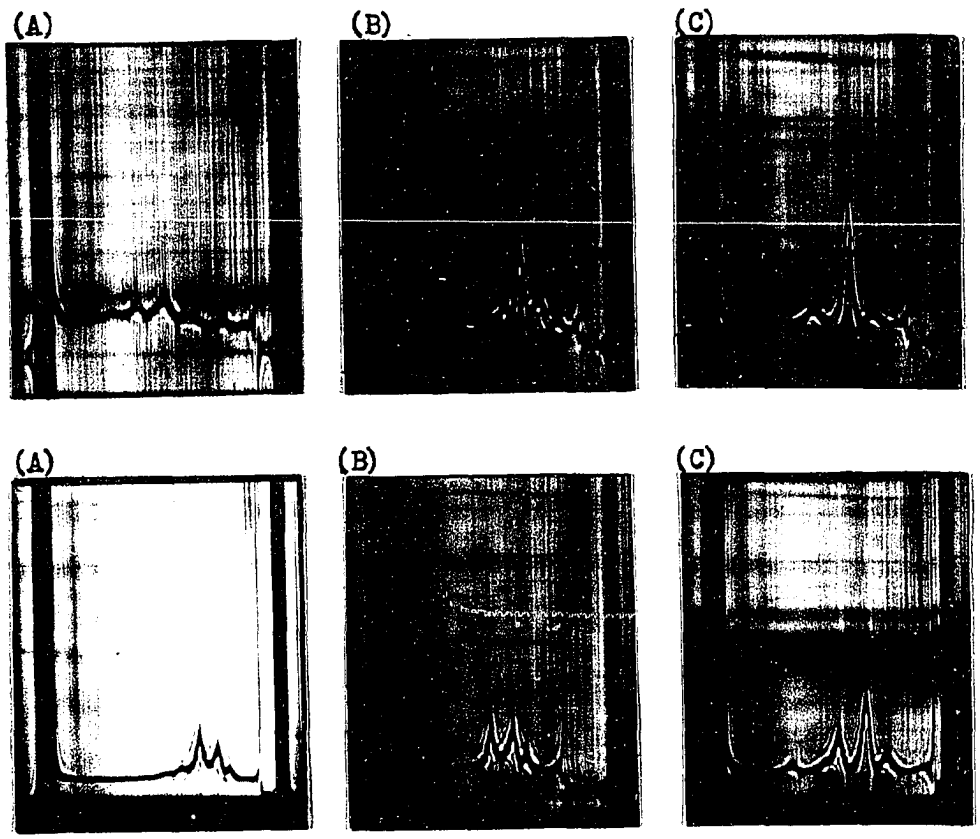
Sedimentation Coefficients of the Ribosomes

Since the sedimentation coefficient depends on the

Figure 3. Effect of magnesium ions on the size distributions of the ribosomes. Sedimentation is from right to left. All centrifugations were performed at 29,500 r.p.m. All pictures were taken at a bar angle of 50°. (A) Standard isolation procedure using 0.01 M tris-magnesium acetate (pH 7.4). (B) Isolation of the ribosomes as in (A) but the final solution was dialyzed versus 0.01 M tris (pH 7.4) and 0.005 M magnesium acetate (top row), or dialyzed versus 0.005 M tris-magnesium acetate (pH 7.4)(bottom row). (C) Ribosomes prepared as in (A) but the final solution was dialyzed versus 0.01 M tris (pH 7.4) and 0.001 M magnesium acetate (top row), or dialyzed versus 0.005 M tris (pH 7.4) and 0.001 M magnesium acetate (bottom row).

Top row : B. *licheniformis*. Sedimentation coefficients : (A) 27, 63, 75, 97, 113; (B) 30, 47, 70, 93; (C) 27, 43, 68.

Bottom row : B. *stearothermophilus* FJW. Sedimentation coefficients : (A) 50, 68, 98, 128; (B) 60, 67, 97, 119; (C) 32, 46, 63, 92.



concentration, it is necessary to obtain the values at infinite dilution by the extrapolation of a plot of s_{obs} versus concentration. Therefore, four runs ranging from OD_{260} of 125 to OD_{260} of 25 were made with the schlieren optics, while three runs ranging from OD_{260} of 0.8 to OD_{260} of 0.25 were made with ultraviolet optics. Unfortunately, attempts to obtain the sedimentation coefficients with ultraviolet optics were not successful because the components were not well resolved and we had great difficulty in making accurate measurements from the tracings obtained with the Joyce-Loebl densitometer. Accordingly, we limited our calculations to the schlieren runs.

It is known that in a multicomponent system, the presence of any one component may affect the sedimenting behavior of another component. This is another reason why values of the sedimentation coefficient must be obtained by extrapolation to infinite dilution so that such effects are eliminated. Moreover, changes in total ribosome concentration may affect reversible interactions between various particles and lead to changes in their relative amounts. In that case, an extrapolation based on total concentration would have to be replaced by one using the concentration of the individual components. In order to check on this possibility, s^{W}_{20} values at infinite dilution were obtained from two different plots. In the one plot, s^{W}_{20} was plotted versus the total ribosome concentration expressed as OD_{260} (the absorbance at 260 mu is due principally to the ribosomal RNA). In the other plot,

s^{w}_{20} was plotted versus the concentration of each component expressed as the area underneath the peak. These two sets of plots are shown in Figure 4.

It can be seen that the two types of plots are essentially identical. This is a good indication that no significant changes occur in the relative amounts of the various ribosomes as a result of progressive dilution.

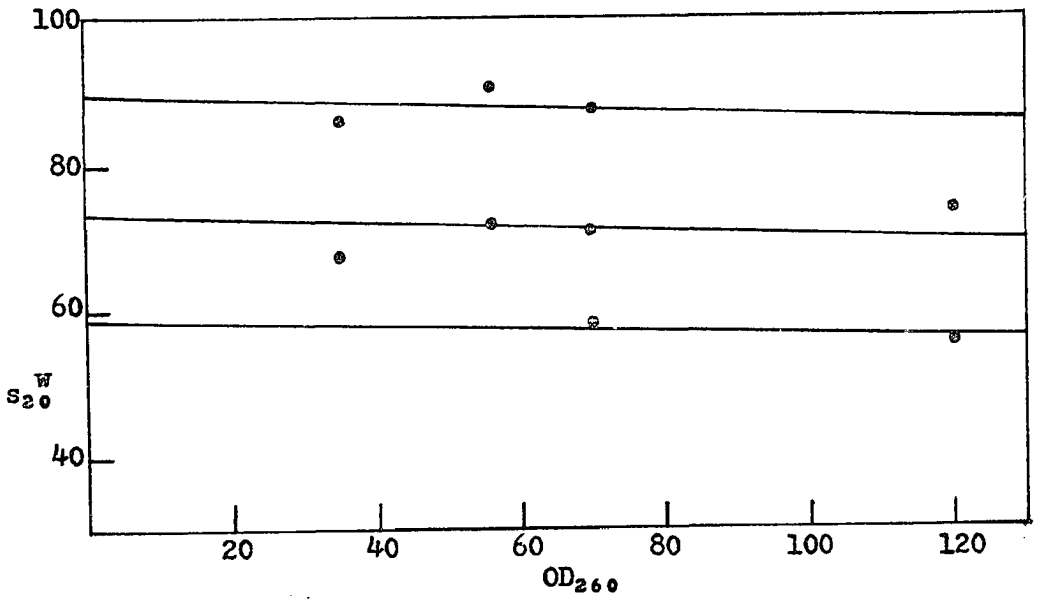
It has been widely accepted that the monomers of ribosomes are of two types. Most bacterial ribosomes are of the 70 S type, while in yeast, higher plants, and animal cytoplasm, the ribosomes are of the 80 S type. From the values that we obtained above, it is clear that the ribosomes from all the six strains of Bacillus, three mesophiles and three thermophiles, belong to the 70 S category. The preparations contained in addition to the monomers also other ribosomes, such as the 30 S and 50 S subunits, or the 100 S dimer, which are similar to those reported for other ribosomes from Bacillus (12,15,24). In all cases, the monomers or larger ribosomes, were predominant.

Bacillus sp.(X-1) contained a component having a sedimentation coefficient of 84 S. It is possible that this component is an association product of two 50 S particles, rather than the dimer of 70 S monomers. Also, one of the components in B. pumilus, 59 S, might be the dimer of 30 S particles, rather than being the 50 S subunit. The value of 90 S for one of the ribosomes of B. pumilus, is rather low for a dimer of 70 S ribosomes. Since

Figure 4. Dependence of the sedimentation coefficients of the ribosomes on concentration.

(a) B. pumilus

1. s^{w}_{20} versus OD_{260}



2. s^{w}_{20} versus Area

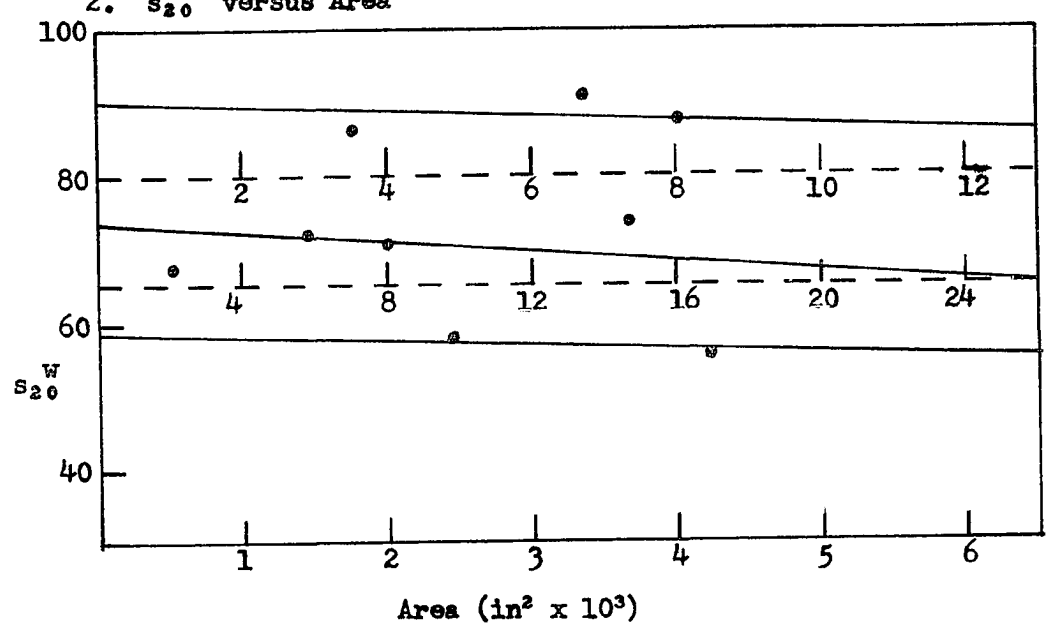
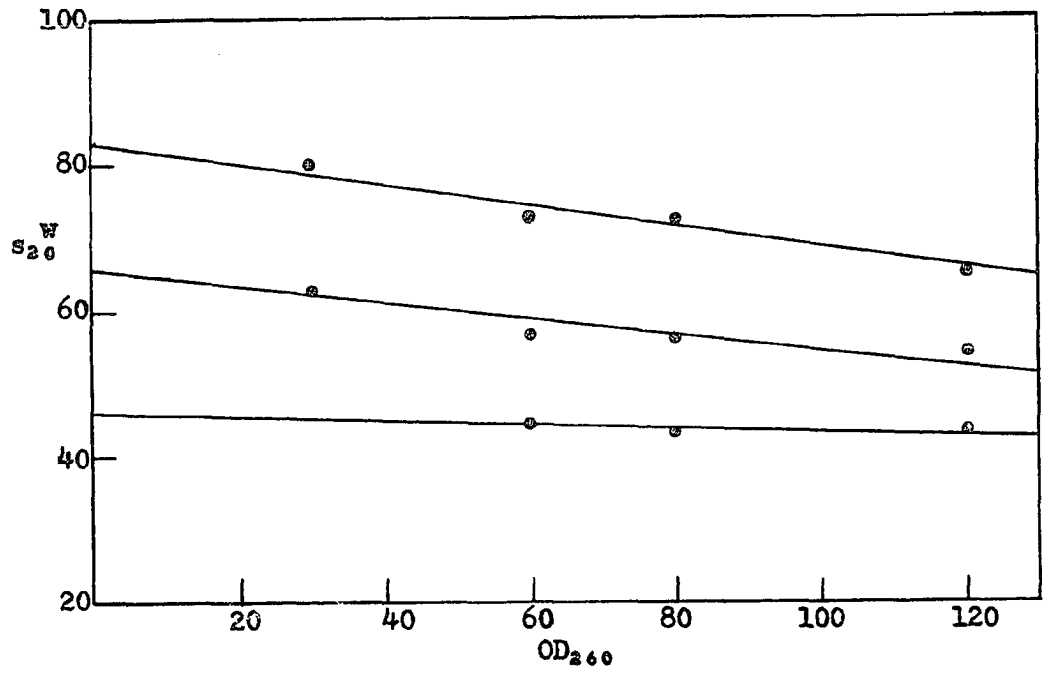


Figure 4.

(b) B. sp. (X-1)

1. s_{20}^{w} versus OD_{260}



2. s_{20}^{w} versus Area

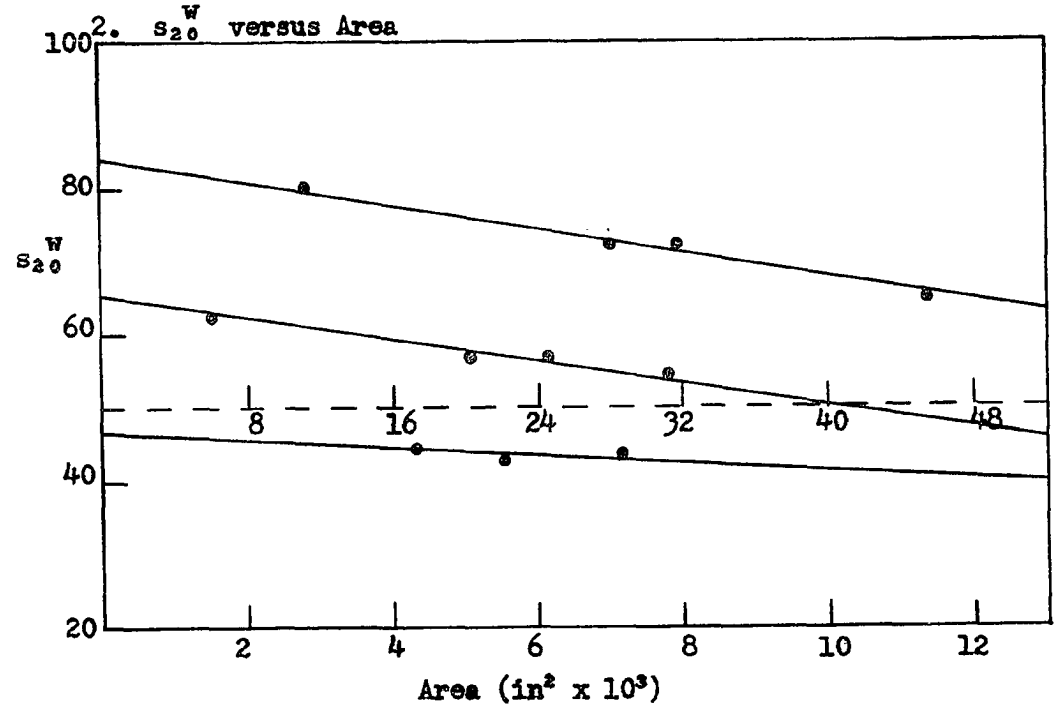
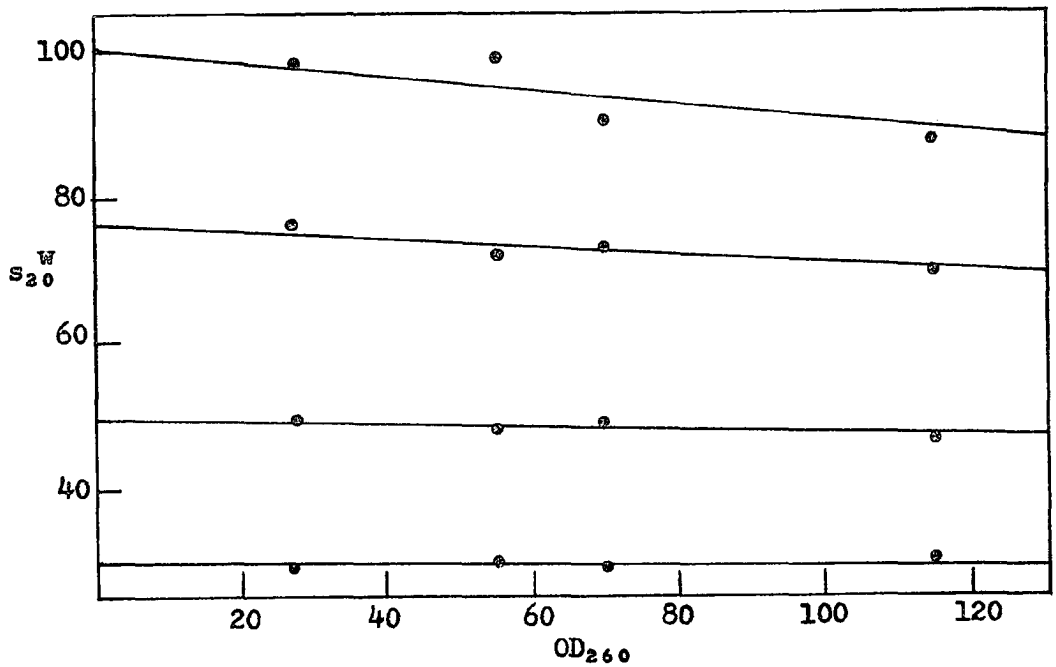


Figure 4.

(c) B. licheniformis

1. s^{w}_{20} versus OD_{260}



2. s^{w}_{20} versus Area

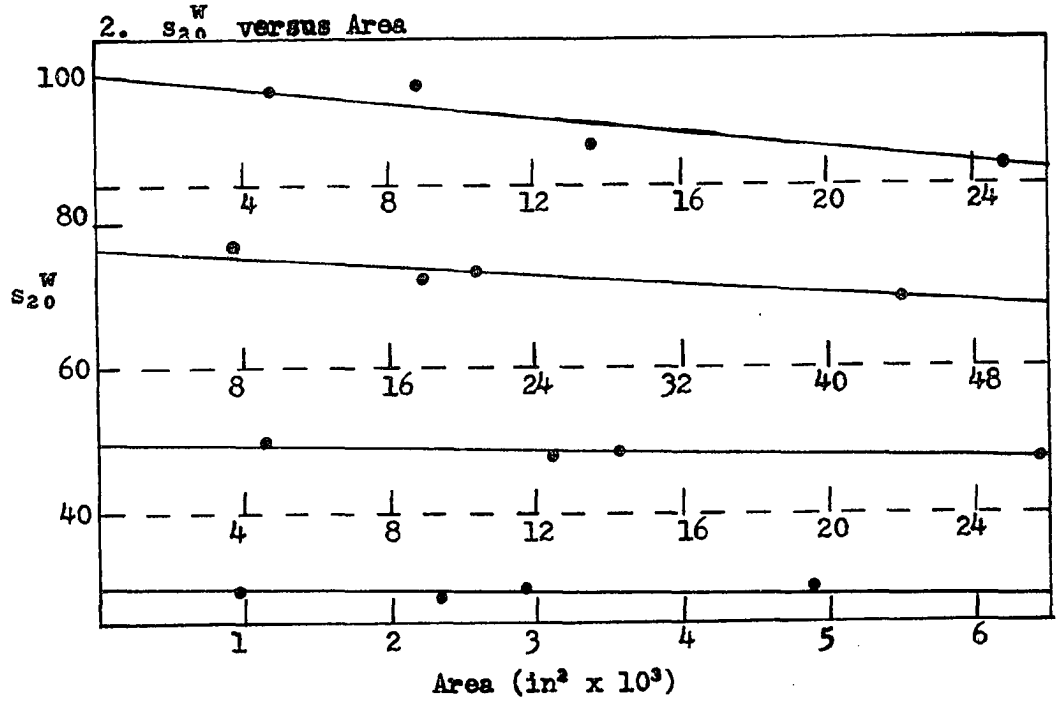
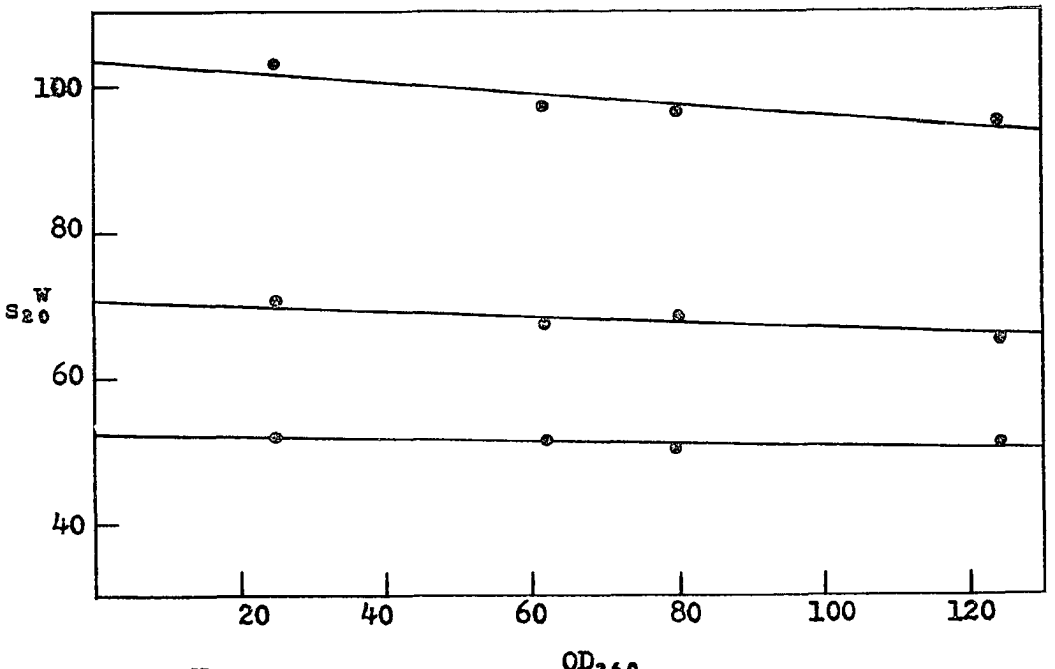


Figure 4.

(d) B. stearothermophilus FJW

1. s^{w}_{20} versus OD_{260}



2. s^{w}_{20} versus Area

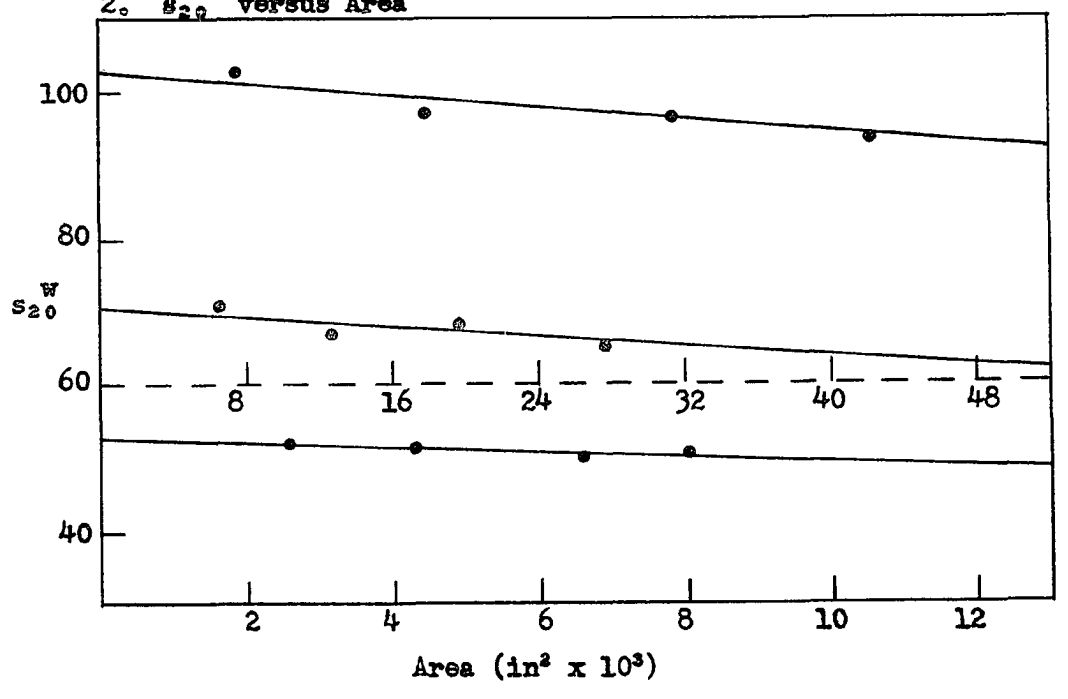
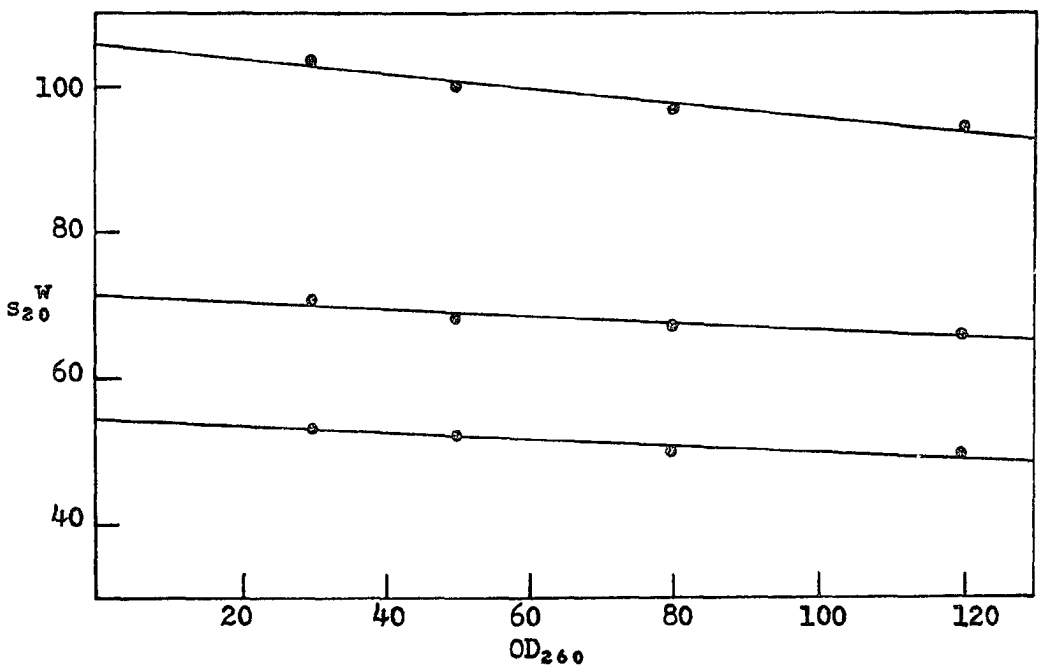


Figure 4.

(e) B. stearothermophilus 10

1. s_{20}^{w} versus OD_{260}



2. s_{20}^{w} versus Area

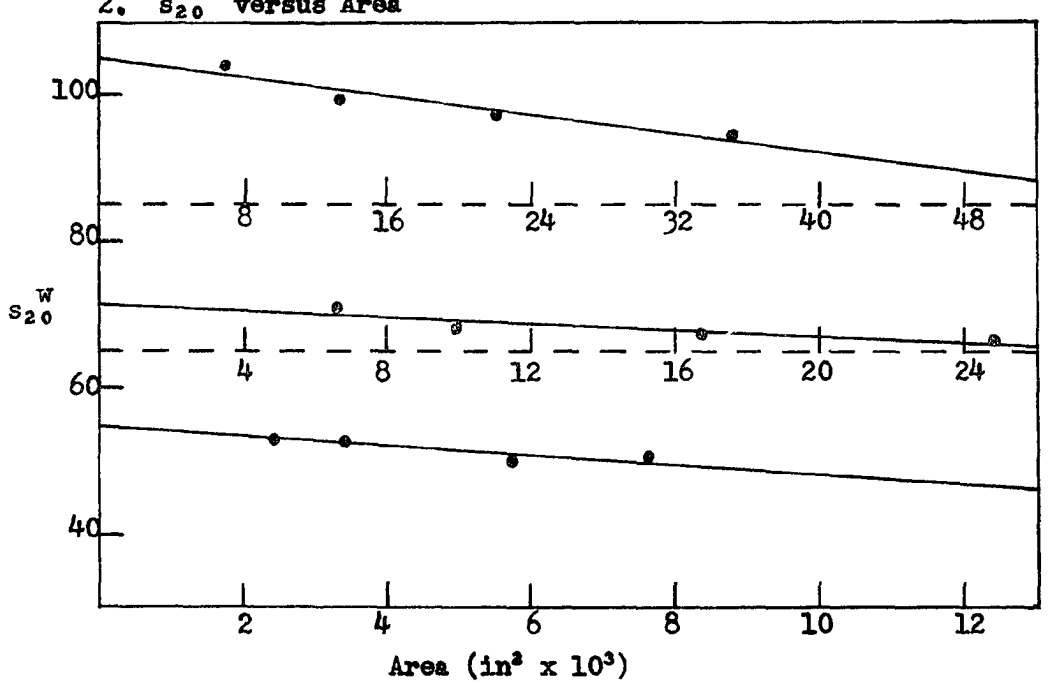
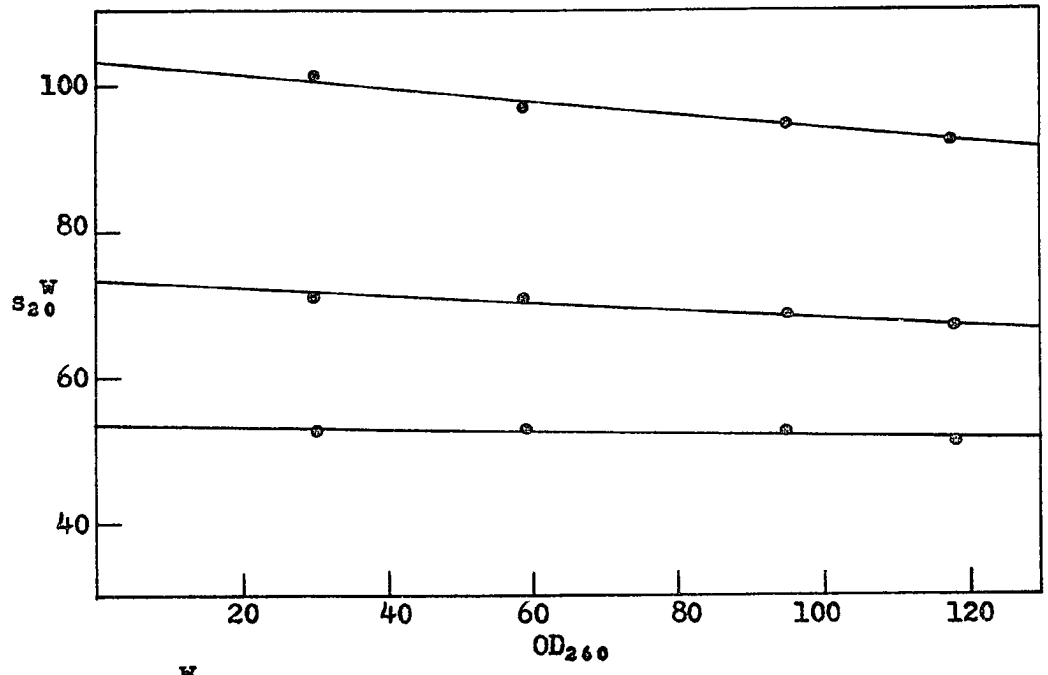


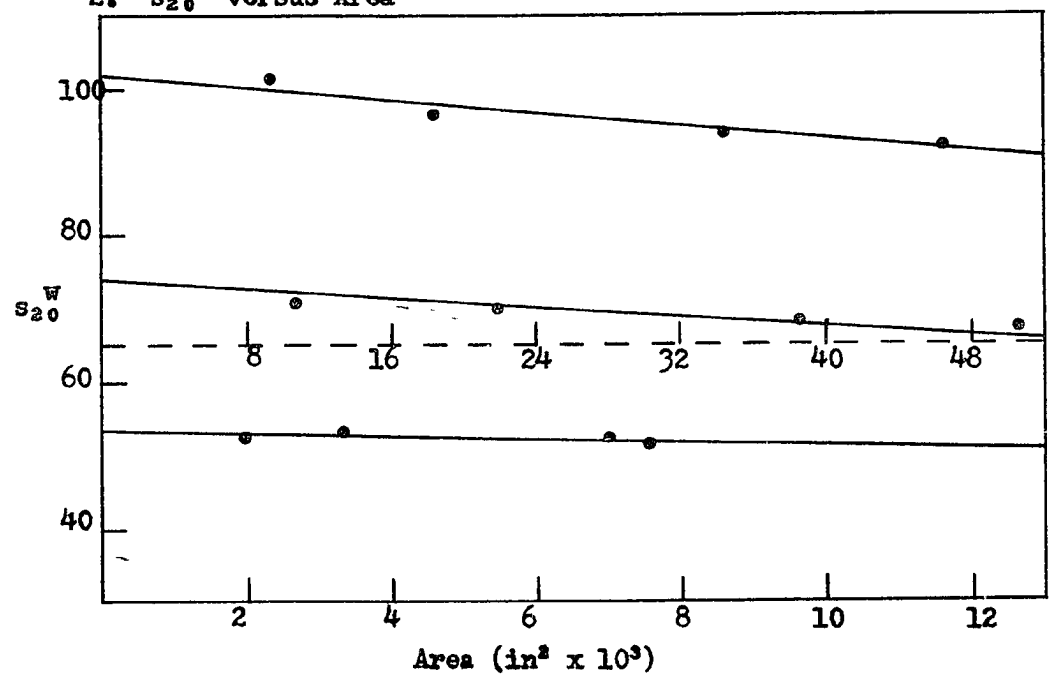
Figure 4.

(f) B. stearothermophilus 2184

1. s_{20}^{w} versus OD_{260}



2. s_{20}^{w} versus Area



the ribosomes from this bacteria gave very poorly resolved ultracentrifuge patterns, it is possible that the sedimenting rate of the larger particles was appreciably slowed down in the presence of the other lighter particles.

Outside of these considerations there seem to be no major differences between the sedimentation coefficients of the ribosomes from the mesophiles and the thermophiles. This refers to both the dependence of s on the concentration and to the extrapolated values of the sedimentation coefficients (see also Tables 1 and 2).

Diffusion Coefficients of the Ribosomes

Since the diffusion coefficient, like the sedimentation coefficient, depends on the concentration, it is also necessary to obtain its value at infinite dilution. For the same reason as mentioned in the discussion of sedimentation coefficients, two sets of plots were made in the same manner as described in the previous section. The results are shown in Tables 1 and 2. Again, since the films from the ultraviolet optics (very dilute solutions) did not lend themselves to accurate measurements, the data are limited to computations using the schlieren runs.

Since the components were not well resolved, the determination of the diffusion coefficients was somewhat more difficult as compared to the determination of the sedimentation coefficients, mostly due to the difficulty involved in the tracing of the

Table 1. Sedimentation coefficients, diffusion coefficients, and molecular weights of the ribosomes from OD_{260} plots.

Organism	s^{W}_{20}(S)	D^{W}_{20} ($cm^2/sec \times 10^7$)	Mol. Wt. ($gm/mole \times 10^{-6}$)
B. licheniformis	29.8	2.84	0.8
	49.5	2.05	1.7
	76.0	2.24	2.4
	100.2	1.50	4.8
B. pumilus	58.6	2.20	1.9
	73.2	2.15	2.4
	89.8	1.36	4.7
B. sp. (X-1)	46.0	-	-
	65.4	2.15	2.2
	82.8	1.50	4.0
B. stearothermophilus FJW	52.2	2.12	1.8
	70.4	2.00	2.5
	103.4	1.58	4.7
B. stearothermophilus 10	54.1	2.65	1.5
	71.3	2.10	2.4
	105.8	1.60	4.7
B. stearothermophilus 2184	53.0	2.25	1.7
	73.0	2.25	2.3
	103.0	1.64	4.5

Table 2. Sedimentation coefficients, diffusion coefficients, and molecular weights of the ribosomes from area plots.

Organism	s^{W}_{20}(S)	D^{W}_{20} ($cm^2/sec \times 10^7$)	Mol. Wt. ($gm/mole \times 10^{-6}$)
B. licheniformis	29.8	2.65	8.0
	49.4	1.95	1.9
	76.0	2.25	2.4
	100.1	1.55	4.6
B. pumilus	58.6	2.05	2.0
	73.7	2.11	2.5
	90.2	1.30	5.0
B. sp. (X-1)	46.6	-	-
	65.2	2.19	2.1
	84.0	1.54	3.9
B. stearothermophilus FJW	52.8	2.26	1.7
	70.1	1.93	2.6
	102.8	1.68	4.4
B. stearothermophilus 10	54.7	2.66	1.5
	71.4	2.10	2.4
	105.1	1.50	5.0
B. stearothermophilus 2184	53.1	2.33	1.6
	74.0	2.25	2.4
	102.0	1.70	4.3

various areas.

As far as the diffusion coefficient is concerned, there appears again to be no major difference between the ribosomes from the mesophiles and those from the thermophiles.

Molecular weights of the Ribosomes

The molecular weight of a macromolecule can be calculated from its sedimentation and diffusion coefficients, using the Svedberg equation, as described before. The results are shown in Tables 1 and 2. The density of the solvent was measured with a Weld pycnometer at 30.0°C, and found to be 0.9963. The density of water at that temperature is 0.9956. Hence the ratio of $\rho_{solvent}/\rho_{H_2O}$ at 30.0°C is 1.0007. Since the solvent is a dilute aqueous solution, this ratio is essentially independent of temperature over a narrow range of temperatures. Consequently, the density of the solvent at 20.0°C can be calculated from this ratio and the known density of water at 20.0°C. The value calculated and used in the Svedberg equation is 0.9989.

The partial specific volume was calculated from the composition of the ribosomes. Stenesh and Yang (13) have shown that all of the ribosomal preparations consist of about 45% RNA and 55% protein. Since the various ribosomal particles are generally quite similar in their content of protein and RNA, the partial specific volume ($\bar{v}$) can be calculated from the above composition data. Assuming a $\bar{v}$ value of 0.74 for protein and

0.57 for RNA (25), the $\bar{v}$ for the ribosomes turns out to be 0.66 ml/gm.

The calculated molecular weights are given in Tables 1 and 2. It can be seen that the 70 S ribosome has a molecular weight of about 2.5 million. This value, and the values for the other ribosomes, are similar to those reported for the ribosomes of E. coli (15). Again, there was no major difference between the molecular weights of the ribosomes from the mesophiles and those from the thermophiles.

The maximum error in the molecular weight for the 70 S particle is estimated as being $\pm$ 3%, based on an estimated maximum error of $\pm$ 0.3% for the sedimentation coefficient and an estimated maximum error of $\pm$ 2.5% for the diffusion coefficient. The maximal error for the other ribosomal particles are of a similar magnitude.

SUMMARY

Ribosomes were isolated from six strains of Bacillus. Three of these were mesophilic strains: B. licheniformis (NRS 243), B. pumilus (NRS 236), and B. sp. (X-1), and three were thermophilic strains: B. stearothermophilus (FJW, 10, 2184).

All the ribosome preparations were shown to contain a ribonuclease activity similar to the one found in association with many other ribosomes.

Studies on the ribosome breakdown showed that the ribosomes from the mesophiles are less stable at temperatures above 60°C than are those from the thermophiles.

The sedimentation coefficients of the ribosomes were determined and the ribosomes were found to have components with sedimentation coefficients of 30, 50, 70 and 100 S, which are similar to those reported for other ribosomes from Bacillus.

Determinations of the diffusion coefficients of the ribosomes were also made.

The molecular weights for the various components of the ribosomes were calculated according to the Svedberg equation, using the data obtained for the sedimentation and diffusion coefficients. The monomers (about 70 S) had a molecular weight of about 2.5 million, which is close to the molecular weight of E. coli 70 S ribosomes.

The ribosomes from the mesophiles did not differ significantly in their physical parameters as compared to the ribosomes from the thermophiles.

BIBLIOGRAPHY

1. Gaughran, E. R. L., Bacteriol. Rev., 11, 189 (1947).

2. Allen, M. B., Bacteriol Rev., 17, 125 (1953).

3. Gaughran, E. R. L., J. Bacteriol., 53, 506 (1947).

4. Koffler, H., Mallett, G. E., and Adye, J., Proc. Natl. Acad. Sci. U.S., 43, 464 (1957).

5. Stenesh, J., and Koffler, H., Federation Proc., 21, 406 (1962).

6. Campbell, L. L., J. Am. Chem. Soc., 76, 5256 (1954).

7. Campbell, L. L., Arch. Biochem. Biophys., 54, 154 (1955).

8. Stenesh, J., and Holazo, A.A., Biochim. Biophys. Acta, 138, 286 (1967).

9. Marmur, J., Biochim. Biophys. Acta, 38, 342 (1960).

10. Arca, M., Calvori, C., Frontali, L., and Tecce, G., Biochem. Biophys. Res. Commun., 10, 117 (1963).

11. Arca, M., Calvori, C., Frontali, L., and Tecce, G., Biochim, Biophys. Acta, 87, 440 (1964).

12. Mangiantini, M.T., Tecce, G., Toschi, G., and Trentalance, A., Biochim. Biophys. Acta, 103, 252 (1965).

13. Stenesh, J., and Yang, C., J. Bacteriol., 93, 930 (1967).

14. Elson, D., Biochim. Biophys. Acta, 27, 216 (1958).

15. Petermann, M. L., The Physical and Chemical Properties of Ribosomes, Elsevier Publishing Co., Amsterdam, 1964.

16. Anfinsen, C.B., and White, F. H., in Boyer, P. D., Lardy, H., and Myrback, K., The Enzymes, vol. V, Academic Press, Inc., New York, 1961, p. 95-122.

17. McDonald, M.R., in Colowick, S. P., and Kaplan, N.O., Methods in Enzymology, vol. II, Academic Press, Inc., New York, 1955, p. 427-436.

BIBLIOGRAPHY

Continued

18. Dickman, S. R., and Trupin, K. M., Biochim. Biophys. Acta, 30, 200 (1958).

19. Wade, H.E., Biochem. J., 78, 457 (1961).

20. Morais, R., and de Lamirande, G., Biochim. Biophys. Acta, 95, 40 (1965).

21. Singer, M.F., and Tolbert, G., Biochemistry, 4, 1319 (1965).

22. Grunberg-Manago, M., and Ochoa, S., J. Am. Chem. Soc., 77, 3165 (1955).

23. Wade, H.E., Lovett, S.,and Robinson, H. K., Biochem. J., 93, 121 (1964).

24. Saunders, G. F., and Campbell, L. L., J. Bacteriol., 91, 332 (1966).

25. Spahr, P.F., J. Mol. Biol., 4, 395 (1962).

VITA

The author was born on June 11, 1943, in Wu Fong, Taiwan. She received her primary and secondary education from the public schools of Taiwan. She attended Tunghai University in Taichung from 1961 to 1965 and graduated with a Degree of Bachelor of Science. She then enrolled at Western Michigan University in the Master's program.