On the Unity of Bacterial Ribosomes

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SUMMARY

Reported sedimentation coefficients of bacterial ribosomes display noticeable variation. Since this might be due to differences in experimental conditions or to actual differences amongst the bacteria this problem needed re-investigation. The sedimentation coefficients of ribosomes from seven widely divergent bacteria (*Streptomyces*, *Acetobacter*, *Pseudomonas*, *Azotobacter*, *Escherichia*, *Streptococcus* and *Bacillus*) were determined by analytical ultracentrifugation. The bacteria were selected to cover nearly the entire range of molar guanine + cytosine content of DNA. Correction for pressure and dilution was always negligible; correction for temperature was eliminated by working directly at 20.0°. The correction for concentration was the most important one. Further reduction to standard conditions (from dilute buffer to water) resulted in a change of about 3%. The corrected sedimentation coefficients, expressed as $(s_{20, w})_0$, were nearly the same for all these bacteria, being in the ranges 29.5 ± 2 , 37.5 ± 2.8 , 56.8 ± 1.9 , 76.7 ± 2.5 , $110.5 \pm 1.6 \times 10^{-13}$ sec. It thus seems unlikely that these coefficients will be useful as an aid in bacterial taxonomy. Disruption of samples of the same bacterial suspension by ultrasonic treatment or with the French pressure cell frequently resulted in quantitative, and sometimes qualitative, differences in the yield of ribosomes.

INTRODUCTION

Living cells appear to contain about five or six types of ribosomal particles, which can be differentiated by their sedimentation coefficient. The ribosomes of *Saccharomyces cerevisiae*, higher plants and animals appear to be slightly larger than those of *Escherichia coli*, since they sediment somewhat faster; the former sediment at about 27, 40, 60, 80 and 120s (Svedberg units), whereas the latter do so at about 20, 30, 50, 70 and 100s. The basis for the widespread opinion that all bacteria would have identical ribosomes resides on the pioneering paper of Schachman, Pardee & Stanier (1952), who discovered that *E. coli*, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Rhodospirillum rubrum*, *Staphylococcus aureus* and *Clostridium kluyverii* contain RNA particles sedimenting at 20–29s, 32–48s and 55–58s (uncorrected). Tissières, Watson, Schlessinger & Hollingworth (1959) established the corrected *s* values of ‘coli’ ribosomes as being $20, 30.6 \pm 1, 50.0 \pm 1, 69.1 \pm 1$ and 100.0 ± 2 . Subsequent measurements have sometimes been quite close to these values, others agree less well, possibly because not all the corrections were applied. From the results of Bolton *et al.* (1959) one can calculate $34.3 \pm 0.9, 53.3 \pm 1.9, 69.4 \pm 5.4$ and 84–100s (partially corrected) depending upon Mg^{2+} concentration. Dagley & Sykes (1958) found 20, 29 and 40s, Elson (1959) found 35 and 52s, whereas Bowen, Dagley & Sykes (1959) and Bowen, Dagley, Sykes & Wild (1961)

mentioned a whole series of values between 17 and 90 s, depending on external conditions. Relatively little information is available about the exact sedimentation coefficients of ribosomes from other bacteria. Corrected *s* values of 10·4, 33·2, 49·7, 54·5, 69·7, 84·8 and 106 were reported by Brown & Rosenberg (1962) for *Sarcina lutea* and 10, 39, 58, 77 and 86 by Gillchriest & Bock (1958) for *Azotobacter vinelandii*. Other values, chiefly uncorrected or partially corrected, have been reported for *Bacillus cereus* (30 and 50 s; Bowen *et al.* 1961), with vegetative forms of *B. subtilis* (33 ± 2 , 50 ± 2 , 68 ± 1 , 99 ± 3 s) and spores of the same strain (27, 33, 50, 70 s; Woese, Langridge & Morowitz, 1960), for *Salmonella typhimurium* (23, 26, 35, 55, 70, 75 and 85 s; Ecker & Schaechter, 1963) and for *Mycoplasma gallisepticum* (17, 38, 58, 76, 84, 117 s; Morowitz *et al.* 1962). Thus some of the above values indeed centre around 20, 30, 50, 70 and 100 s, but others seem considerably divergent. This casts some doubt on the validity of the general belief that bacterial ribosomes are identical. Alternatively, on the basis of the above numbers, it is not excluded that differences may exist between ribosomes of different bacterial taxa which might have taxonomic implications. It therefore seemed interesting to re-investigate the sedimentation coefficients of bacterial ribosomes by using several widely divergent bacteria, but with the same experimental conditions and the same analytical ultracentrifuge. Two different methods of cell disruption were used (ultrasonic treatment and French pressure cell) in order to increase the chance of detecting all ribosomal types.

METHODS

Organisms and culture conditions

Most strains were from the collection in our department; bacteriological controls and precautions were taken to ensure that only pure cultures were used.

Streptomyces lavendulae strain 265 was grown in a liquid medium containing (% w/v): 0·5, peptone; 0·5, glucose; 0·25, yeast extract (Ned. Gist- en Spiritusfabriek, Brugge). 200 ml. volumes of medium were shaken in 1 l. Erlenmeyer flasks for 24 hr at 30°. Yield: 7·5 g. wet wt. organisms/l. culture medium.

Acetobacter aceti (*xylinum*) strain NCIB 8747 was grown in Roux flasks on 150 ml. solid medium containing (% w/v): 10, glucose; 1, yeast extract; 3, CaCO₃; 2·5, agar. Yield after 2 days at 30°: 1 g. wet wt. organisms/flask.

Pseudomonas fluorescens strain 488 (obtained through the courtesy of Dr O. Lysenko Prague) was grown for 24 hr at 30° in Roux flasks with 150 ml. solid medium containing (% w/v): 1, yeast extract; 1, glucose; 2, CaCO₃; 2·5, agar. Yield: 1 g. wet wt. organisms/flask.

Escherichia coli strain 101 was grown for 21 hr at 30° in Roux flasks on solid medium containing (% w/v): 0·5, peptone; 0·25, yeast extract; 0·5 glucose; 2·5, agar. Yield: 700 mg. wet wt. organisms/flask.

Azotobacter vinelandii strain 7492 (obtained through the courtesy of Professor J. Voets, Department of Soil Microbiology, Ghent) was grown with continuous shaking for 24 hr at 30° in a liquid medium containing (% w/v): 1, glucose, 0·1, K₂HPO₄; 0·25, KH₂PO₄; 0·25, NaCl; 0·25, MgSO₄·7H₂O; 0·125, CaSO₄; 0·012, Fe₂(SO₄)₃; in tap water. Yield: 8·2 g. wet wt. organisms/l. The organisms were also grown on the same medium, but solidified with agar, in Roux flasks for 24 hr.

Bacillus cereus strain BCV was grown for 2 days at 30° in Roux flasks on a solid medium containing (% w/v): 0.5, peptone; 0.25, yeast extract; 0.5, glucose; 0.01, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.036, KH_2PO_4 ; 0.052, Na_2HPO_4 ; 2.5, agar. Yield: 1.7 g. wet wt. organisms/flask.

Streptococcus faecalis (obtained through the courtesy of Professor J. Voets, Department of Soil Microbiology, Ghent) was grown for 53 hr at 30° in flasks filled to the neck with a liquid medium containing (% w/v): 1, yeast extract; 0.01, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.054, KH_2PO_4 ; 0.085, Na_2HPO_4 ; in tap water. The medium was sterilized at 120° for 20 min., filtered and sterilized again. To it were added sterilized solutions of glucose (to final 1 %) and Liebig meat extract (to final 0.5 %). Yield: 1.36 g. wet wt. organisms/l.

Preparation of cell-free extracts

Organisms were harvested by centrifugation and washed two or three times by suspending in TMS buffer pH 7.1 (0.01 M-tris, 0.004 M-succinic acid; 0.005 M- MgSO_4). The same buffer was used in all the work. The organisms were finally suspended at a concentration of about 100–200 mg. wet wt. organisms/ml. buffer. Half of each suspension was disrupted in a French pressure cell with Wabash hydraulic press (American Instruments Co., Silver Spring, Md., U.S.A.) at 7600 lb./in.²; the other half was treated in the 10 kcy., 250 W. Raytheon Sonic Oscillator at 4° in H_2 atmosphere. In the former procedure the suspension was usually passed twice through the pressure cell, which sufficed for nearly complete breakage. With the Raytheon instrument the treatment was continued until nearly all the organisms were broken. In both cases the disruption was followed by phase-contrast microscopy. After disruption all suspensions were centrifuged at 13,000 *g* for 10–15 min. at 4° to eliminate unbroken organisms and large pieces of debris. In many cases a minute crystal of crystalline deoxyribonuclease (Worthington, Freehold, N.J., U.S.A.) was added to the supernatant fluid, which was then incubated for about 1 hr at 22° and then at once subjected to ultracentrifugal analysis.

Ultracentrifugation

Analytical ultracentrifugation was done in a Spinco Ultracentrifuge, model E, with Rotor Temperature Indicator Control (RTIC) at 20.0°, schlieren optics, rotor An-E at 37,020 rev./min. and 18 mm. optical cells (one standard and one wedge). Each optical cell contained 1.7 ml. of crude cell-free extract. The rotor and the optical cells were equilibrated thermally with the RTIC system in the centrifuge before each run. About 7–10 min. after the desired speed had been reached, photographs were taken, at 65° bar angle, every 2 min. with 4–7 sec. exposure time, on Kodak Metallographic plates. Measurements of the position of the schlieren peaks on the photographic plates were at first determined with a Leitz TP comparator (to 0.001 mm.), but this was found too time-consuming for routine use. Later we used our own method with a photographic enlarger, which projected the picture, 10 times enlarged, on a sheet of millimetre paper (28 × 32 cm.). Both the millimetre paper and the position of the enlarger were calibrated so that the position of the schlieren peaks in the rotor cell could be determined directly, with an accuracy of 0.5 mm. on the paper, which was equivalent to 0.025 mm. in the rotor. This method was found to be reproducible, and faster and more convenient than the one with the comparator. A similar method was described by Schachman (1957).

RESULTS

Corrections to be applied to experimental s values

Correction for the effect of pressure and dilution due to the pressure gradient in the rotor cell and its sector shape. Elias (1961) pointed out that this correction can be found by plotting $(s_c)_{app.}$ against $1 - r_m^2/\bar{r}^2$, in which $(s_c)_{app.}$ is the experimentally determined sedimentation coefficient, r_m the distance of the meniscus to the rotor axis (in cm.) and $\bar{r}$ the average distance of the schlieren peaks to the rotor axis (in cm.). The value obtained by correction for these effects is called s_c . Figure 1 illustrates one case and shows that this correction did not apply in our case. This was found to be so in every experiment.

Fig. 1. The sedimentation coefficients of bacterial ribosomes are independent of the effect of pressure and dilution. Example of an extrapolation with ribosomes of *Acetobacter aceti* (*xylinum*). $(s_c)_{app.}$ was determined as described in the text and calculated according to Svedberg & Pedersen (1940). r_m and $\bar{r}$ were determined with a photographic enlarger.

Correction for the effect of temperature. Sedimentation coefficients are often determined at 4° or at least below room temperature. Since ribosomes are quite stable for several hours at room temperature, we eliminated the temperature correction by measuring directly in the standard conditions of 20.0°. The speed of 37,020 rev./min. was selected to make the duration of the experiment as short as possible and still to allow measurable speeds of sedimentation.

Correction for the effect of concentration. This was found to be most important. From each crude extract dilutions of 3/4, 1/2, 1/4 and usually also 1/8 in TMS buffer were made and the sedimentation coefficients determined. These values were extrapolated to zero concentration. The extrapolated value will be called $(s_{20, TMS})_0$. Figure 2 illustrates one experiment. In this particular case the difference between the sedimentation coefficient s_c in the crude extract and $(s_{20, TMS})_0$ at infinite dilution amounted to 31 %. With the other bacteria, differences of 10–38 % were observed. This means that quite considerable errors can be introduced when this correction is not taken into account. For example, direct estimation of a ribosome in bacterial

extract always gives too low s values, with the correspondingly incorrect s nomenclature. Also, when the concentration of the bacterial extract is rather high, the s_c of one type of ribosome may be lower than the $(s_{20,w}^{\text{TMS}})_0$ value of the following one, resulting in its classification in the wrong group. The larger the ribosome, the more pronounced is this effect.

Not infrequently one or two of the ribosomal types were only visible at the highest concentration and practically disappeared on dilution. The $(s_{20,w}^{\text{TMS}})_0$ value could thus not be determined by extrapolation. A fair approximation, however, was given by the following procedure. It had been observed that, at each concentration c , a linear relationship existed between s_c and $(s_{20,w}^{\text{TMS}})_0$ for all the ribosomes

Fig. 2

Fig. 2. The dependence of the sedimentation coefficients of bacterial ribosomes on the concentration. The crude extract of *Streptococcus faecalis* was prepared from a suspension with 112 mg. of living cells/ml. TMS buffer (equals concentration $c = 1$ in the graph). The extrapolation of s_c to $c = 0$ yields $(s_{20,w}^{\text{TMS}})_0$.

Fig. 3

Fig. 3. Linear relationship between $(s_{20,w}^{\text{TMS}})_0$ and s_c of several ribosomes from the same preparation. The results are taken from Fig. 2. The curve $c_{\text{exp.}}$ represents the actual relationship found with s_c at concentration c (crude extract of a suspension of 112 mg. of living cells/ml. TMS buffer). Smaller concentrations yield curves which approach the theoretical one at c_0 , where s_c equals $(s_{20,w}^{\text{TMS}})_0$.

of each extract (Fig. 3). In case only a very small amount of any one ribosome was present in an extract, a plot of s_c against the $(s_{20,w}^{\text{TMS}})_0$ values of the other ribosomes was made, upon which the $(s_{20,w}^{\text{TMS}})_0$ value of the ribosome present in trace amount could be deduced from its s_c value in the crude extract. The results obtained by this procedure corresponded closely to the $(s_{20,w}^{\text{TMS}})_0$ value of the same ribosome from other bacteria, obtained by the regular extrapolation (for examples see Table 1).

Reduction to standard conditions. This is usually carried out by means of the formula of Svedberg & Pedersen (1940):

$$(s_{20,w})_c = (s_{t,\text{solv.}})_c \frac{\eta_{t,w}}{\eta_{20,w}} \frac{\eta_{t,\text{solv.}}}{\eta_{t,w}} \frac{1 - V_{20,\text{solv.}} \cdot \rho_{20,w}}{1 - V_{t,\text{solv.}} \cdot \rho_{t,\text{solv.}}},$$

This equation has been verified experimentally for solutions of proteins in buffer and for synthetic high polymers. According to Elias (1961) it is applicable only in narrow ranges of temperature and with bad approximation. In our case we have only to reduce $(s_{20, \text{TMS}})_0$ from buffer to water and the above equation becomes:

$$(s_{20, w})_0 = (s_{20, \text{TMS}})_0 \frac{\eta_{20, \text{TMS}} (1 - V_{20, \text{TMS}} \cdot \rho_{20, w})}{\eta_{20, w} (1 - V_{20, \text{TMS}} \cdot \rho_{20, \text{TMS}})},$$

in which $(s_{20, w})_0$ is the value reduced to water at 20° and at infinite dilution; $\eta_{20, \text{TMS}}$ is the viscosity of the TMS buffer at 20°; $\eta_{20, w}$ is the viscosity of water at 20°; $V_{20, \text{TMS}}$ is the partial specific volume of the ribosomes at 20° in TMS buffer; $\rho_{20, \text{TMS}}$ is the specific gravity of the TMS solution at 20°; $\rho_{20, w}$ is the specific gravity of water at 20°. It can be expected theoretically that $\eta_{20, \text{TMS}}/\eta_{20, w}$ and $\rho_{20, w}/\rho_{20, \text{TMS}}$ will be close to unity. In fact, the former ratio was experimentally found to be

Table 1. *Some examples of corrected sedimentation coefficients, expressed as $(s_{20, \text{TMS}})_0 \times 10^{13}$ sec of ribosomal particles of several bacteria*

(Values in parentheses are from particles present in very small amount only in the highest concentration of crude extract; they could not be determined by extrapolation but the $s_0 - (s_{20, \text{TMS}})_0$ plots were used, as exemplified by Fig. 8. The percentage guanine + cytosine of *Pseudomonas* and *Acetobacter* was determined in the laboratory; the other values are taken from the literature.)

Strain	Molar percentage of guanine + cytosine in DNA	Method of disruption	$(s_{20, \text{TMS}})_0 \times 10^{13}$ sec.							
			(28.5)	36.6	(56)	74.4	—	—	—	—
<i>Streptomyces lavendulae</i>	74	French pressure cell								
<i>Acetobacter aceti</i> (<i>xylinum</i>)	61	French pressure cell	(28.3)	—	53.8	72.5	(111)	—	—	—
		Raytheon	—	—	54.0	74.0	112.2	139	168	(188)
<i>Pseudomonas fluorescens</i>	60	Raytheon	28.6	38.0	(58.5)	74.1	(107)	—	—	—
<i>Azotobacter vinelandii</i>	57	Raytheon	—	(37.5)	—	74.5	—	—	—	—
		Raytheon	27.3	35.5	54.0	72.7	108.5	—	—	—
<i>Escherichia coli</i>	50	French pressure cell	28.5	35.3	55.0	74.3	106.7	—	—	—
		Raytheon	(26.2)	37.7	54.7	75.8	107.0	—	—	—
<i>Streptococcus faecalis</i>	34	French	—	34.0	53.0	72.7	106.9	—	—	—
		pressure cell	—	37.6	55.3	73.8	105.3	—	—	—
		Raytheon	—	—	54.1	74.5	105.7	—	—	—
<i>Bacillus cereus</i>	33	French pressure cell	28.7	33.6	52.6	76.4	—	—	—	—
		Raytheon	28.7	36.9	54.2	75.1	108.5	—	—	—

1.027 by using an Oswald viscosimeter and $\rho_{20, \text{TMS}}$ was determined with a pycnometer to be 1.0023. On the assumption that the partial specific volume of the ribosomes was 0.66, identical in TMS buffer and water and identical for all types of ribosomes, it was calculated that $(s_{20, w})_0 = 1.035(s_{20, \text{TMS}})_0$.

The corrected sedimentation coefficients of bacterial ribosomes

Some representative results are collected in Table 1. The average $(s_{20, \text{TMS}})_0$ values (expressed as Svedberg units) were calculated to be: 28.5 ± 2 , 36.3 ± 2.7 , 54.4 ± 1.8 , 74.2 ± 2.4 , 106.9 ± 1.6 , or, reduced to standard conditions as $(s_{20, w})_0$: 29.5 ± 2 , 37.5 ± 2.8 , 56.3 ± 1.9 , 76.7 ± 2.5 , 110.5 ± 1.6 . The complete set of ribosomes was not always detectable with every strain. For example there appeared to be no 110 s ribosomes in *Streptomyces*, no 37 s ribosomes in *Acetobacter* and sometimes no 29 and 37 s ribosomes in *Streptococcus*. It is very likely that each strain had indeed the complete set, but that some of the ribosomal types were occasionally present in very small concentrations. Furthermore, it is well known that the

Fig. 4. Comparison of schlieren patterns of ribosomes from cells broken with the French pressure cell and by ultrason. Methods, see text. For each strain two pictures are given: the left one was made with the crude extract from cells disrupted by ultrason, the right one with a sample of the same cell suspension disrupted with the French pressure cell. Tracings do not represent pictures taken at the same time but are selected to show all the peaks. The suspensions of intact cells used for disruption contained, for *Acetobacter aceti* (*xylinum*): 124 mg. of wet wt. cells/ml.; *Bacillus cereus*: 210 mg. of wet weight cells/ml.; *Escherichia coli*: 104 mg. of wet wt. cells/ml.; *Streptococcus faecalis*: 112 mg. of wet wt. cells/ml. The numbers accompanying the peaks represent the extrapolated sedimentation coefficients at infinite dilution, expressed as $(s_{20, \text{TMS}})_0$.

relative amounts of the different ribosomes change with the physiological state of the organisms (young cells usually having more of the smaller ribosomes and resting cells mainly the larger ones) and depend on the composition of the growth medium (particularly the Mg^{2+} concentration).

It will be seen from Table 1 and Fig. 4 that *Acetobacter aceti* (*xylinum*) released large particles (139, 168, 188 s) which were not encountered with other strains. It is possible that these were polysomes, but we held it to be far more likely that they represented fragments of the cell hull, which is indeed rather unusual in these bacteria, since it contains much cellulose and other polysaccharides. We showed that purified cell hulls of this strain which had been prepared with the French pressure cell released identical large particles when treated with the Raytheon instruments.

Effect of growth conditions and physiological state of the organisms. The effect of both factors on the relative amounts of ribosomes is well known. However, they appear to have little effect on the sedimentation coefficients. In spite of the fact that the growth conditions varied greatly (some organisms were grown on solid media, others in liquid ones; the amount of Mg^{2+} added varied between 0 and 0.25 %, w/v) and that the physiological state of the organisms was different (age of the cells between 21 and 53 hr; variation from early exponential to stationary phase), the sedimentation coefficients are nearly identical. An experiment with *Azotobacter vinelandii* of the same age, grown either on solid or in liquid medium, showed that the relative amounts of the ribosomes were different, as expected, but that the sedimentation coefficients were nearly indistinguishable.

As the Mg^{2+} concentration of the suspending medium is of primary importance, we selected 0.005 M- $MgSO_4$, because both the large and the small ribosomes are detectable under these conditions (see Bolton *et al.* 1959).

Effect of the method of cell disruption on the amount and the nature of the ribosomes released. With some organisms (*Streptomyces*, *Pseudomonas*, *Azotobacter*) there was hardly any difference between the schlieren pictures of cells broken by either method used. With other organisms (see Fig. 4) quite often there were more of the larger ribosomes when the cells had been disrupted with the Raytheon instrument and more smaller ones when they had been treated with the French pressure cell. An unusual phenomenon was observed with *Escherichia coli* disrupted with the French pressure cell, namely, a broad peak which extended from 54.0 to 72.7 s, as if a family of ribosomes were present.

DISCUSSION

The results show that the ribosomes appear to be identical, or nearly identical, in the examples of seven widely divergent genera of bacteria investigated. It thus seems likely that the s values will be the same for most, if not all, bacteria, including the actinomycetes. If there are small differences, they fall within the experimental error. There seems to be little hope that ribosomal sedimentation coefficients will be of use in bacterial taxonomy for distinguishing between several groups. In our conditions the s values were: 29, 37, 56, 77, 110. The three middle values are near to the values reported by Gillchriest & Bock (1958) for *Azotobacter*, by Morowitz *et al.* (1962) for *Mycoplasma* and by Ecker & Schaechter (1963) for *Salmonella*; they agree less well with the results of other authors. These 37, 56 and 77 s peaks correspond to the 30, 50 and 70 s ribosomes of Tissières *et al.* (1959). Our 110 s peak corresponds to the 100 s ribosomes of Tissières *et al.* (1959); it is the dimer of the 77 s particle (Huxley & Zubay, 1960) and is regarded as inert ribonucleoprotein (McCarthy, 1960). Our 29 s peak is probably similar to the 20 s particle of Tissières *et al.* (1959) and to the 25 s particle of Ecker & Schaechter (1963) and Takai, Oota & Osawa (1962), which was shown by the latter authors to be a protein-rich RNA-poor ribosome-like particle. In our experiments we did not detect the 85 s peak, in agreement with other authors (Tissières *et al.* 1959; Dagley & Sykes, 1958; Elson, 1959; Woese *et al.* 1960). In some cases it was detected as a very small peak (Bowen *et al.* 1961; Brown & Rosenberg, 1962). A distinct 85 s peak was observed with *Escherichia coli* (Bolton *et al.* 1959), *Salmonella* (Ecker & Schaechter, 1963) and *Mycoplasma*

(Morowitz *et al.* 1962). It should be remembered, though, that the 85s value may be too small, since it was usually reported uncorrected. According to Huxley & Zubay (1960) the 85s particle is the dimer of the 56s particle and, since we found the latter to be identical in all strains investigated, there seems no reason to believe that its dimer would vary in the bacterial world.

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