The Inhibition of Ribosomal Ribonuclease by Bacterial Ribosomes

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1. A comparison has been made between the ribonuclease activities of untreated ribosomes from *Escherichia coli* B and *Pseudomonas fluorescens* and the activities of ribosomes on to which ribosomal ribonuclease from *E. coli* B has been adsorbed. 2. The normal ribosomes from both species were stable in 5–10 mM-Mg²⁺ (I₀-16) at pH 6. The RNA in ribosomes from *Ps. fluorescens* was attacked by the adsorbed ribonuclease under these conditions, whereas the ribosomes from *E. coli* B were able to adsorb and inhibit this enzyme. 3. Inhibition was also observed with ribosomes from *Aerobacter aerogenes*, *Proteus vulgaris* and two other strains of *E. coli*. It was not observed in ribosomes from three species of *Pseudomonas*. 4. The inhibition depended on the integrity of the ribosomes and was not observed under conditions of low Mg²⁺ concentration that cause irreversible degradation into more slowly sedimenting particles.

Until recently it was generally accepted that bacterial ribosomes contain a pancreatic-type ribonuclease (Elson, 1959; Spahr & Hollingworth, 1961; Wade, 1961). The evidence for the location of this enzyme in ribosomes rested mainly on its exclusive location in the '30s' component (Spahr & Hollingworth, 1961; Tal & Elson, 1963).

More recently the importance of ribonuclease to ribosome function has been questioned by its absence from ribosomes of *Pseudomonas fluorescens* (Wade & Robinson, 1963) and a strain of *Escherichia coli* (Cammack & Wade, 1965). Also, there are indications that ribonuclease is not exclusively located in ribosomes but is also present in the bacterial membranes, accounting for their destructive effect on ribosomes (Tani & Hendler, 1964; Wade & Robinson, 1965) and for the rapid loss of ribonuclease from *E. coli* during spheroplast formation (Neu & Heppel, 1965). The earlier view that the enzyme becomes adsorbed on to the surface of ribosomes during their isolation has now been revived (Neu & Heppel, 1964).

The availability of ribonuclease-deficient ribosomes from *Ps. fluorescens* provided an opportunity to test the adsorption theory by comparing the properties of ribonuclease-containing ribosomes from *E. coli* B with ribonuclease-deficient ribosomes from *Ps. fluorescens* on to which ribosomal ribonuclease from *E. coli* B had been adsorbed. The results brought to light an inhibition of this enzyme by ribosomes from *E. coli*, *Aerobacter aerogenes* and *Proteus vulgaris* that was not displayed by ribosomes from three species of *Pseudomonas*.

MATERIALS AND METHODS

Cultural conditions. Rapidly dividing bacteria from the genera *Pseudomonas*, *Aerobacter*, *Escherichia* and *Proteus* (Table 1) were grown in defined media by continuous culture (Wade & Robinson, 1965).

Isolation of normal ribosomes. A suspension (10%, wet wt./vol.) of disrupted bacteria (Hughes, 1951) in 0.145 M-NaCl–5 mM-MgCl₂, pH 6–6.5, was fractionated by differential centrifugation to yield a purified (twice-washed) ribosome preparation identified as R8 in a previous paper (Wade & Robinson, 1965).

Extraction of ribosomal ribonuclease. Ribosomes (2–3 g. wet wt.) from *E. coli* B or *Ps. chlororaphis* were suspended in a solution of 0.145 M-NaCl–5 mM-MgCl₂ (total volume 60 ml.), mixed with 120 ml. of 0.15 M-veronal–acetate buffer, pH 7.5 (Roth, 1954), and 20 ml. of 0.2 M-EDTA, pH 7.5, and then incubated at 37° for 40 min. (*E. coli* B) or 3 hr. (*Ps. chlororaphis*). Insoluble protein was removed by centrifuging at 2–4° and the supernatant was dialysed against water, freeze-dried and stored at 2–4°. The product was taken up into 20 ml. of a solution of 0.145 M-NaCl–5 mM-MgCl₂, adjusted to pH 7 with Na₂CO₃ and centrifuged to remove insoluble material. The solutions from the two species contained 5.5 and 2.8 mg. of protein/ml. respectively.

Ribonuclease activity was measured by incubating (at 37°) a sample with ribosomal RNA (3 mg./ml.) in 90 mM-veronal–acetate buffer, pH 6 (Roth, 1954), containing MgCl₂ (1 mM) and NaCl (I₀-16), and following the release of acid-soluble nucleotides as described below. One unit of activity (ribonuclease unit) is that amount of enzyme which liberates nucleotides at the rate of 1 μmole/hr. The solutions of soluble ribosomal protein from *E. coli* B and *Ps. chlororaphis* contained 300–350 and 30 ribonuclease units/ml. respectively.

Adsorption of ribonuclease from *E. coli* B on to ribosomes. Ribosomes (about 1 g. wet wt.) from *E. coli* B or *Ps. fluorescens* were suspended in a solution of 0.145 M-NaCl-5 mM-MgCl₂, pH 6-6.5, in a total volume of 21 ml., treated with 9 ml. of a solution of soluble ribosomal protein from *E. coli* B that contained 300-350 ribonuclease units/ml., and centrifuged at 85000g for 7.5 hr. at 2-5° in the no. 30 rotor of the Spinco model L centrifuge to sediment the ribonuclease-adsorbed ribosomes. About 80% of the enzyme was adsorbed on to ribosomes from *Ps. fluorescens* and about 98% on to ribosomes from *E. coli* B.

Autodegradation of ribosomes between pH 4 and 9. The ribosomes were suspended (about 15 mg. dry wt./ml.) in 0.145 M-NaCl-5 mM-MgCl₂, pH 6-6.5, and diluted with 2.3 vol. of 0.13 M-veronal-acetate buffer (Roth, 1954), I.O-16, containing EDTA (28.5 mM), or with 4 vol. of 0.11 M-veronal-acetate buffer, I.O-16, with or without the further addition of MgCl₂ (4 mM) (Fig. 1). The suspensions were incubated at 37° and samples (0.05 ml.) were taken to determine acid-soluble nucleotides as described below.

Estimation of RNA degradation. Acid-soluble nucleotides formed during the breakdown of RNA were extracted by delivering samples (0.05 ml.) on to strips of filter paper and immersing them in 10 ml. of 0.1 N-HClO₄ at 20°. The extent of breakdown of endogenous (ribosome-bound) RNA was assessed from the extinction at 260 mμ compared with the maximum extinction obtained from a sample that had been hydrolysed with alkali (Wade, 1961).

The rate of breakdown of exogenous (added) RNA was followed in the same way except that the acid extraction was carried out at 2-5°. The lower temperature was necessary because free RNA is more readily hydrolysed to acid-soluble fragments by 0.1 N-HClO₄ than is ribosome-bound RNA. In those instances where RNA degradation is recorded as acid-soluble nucleotide released, a mean molar extinction (ϵ) 10⁴ was used to calculate the approximate molarity of the nucleotides.

Preparation of RNA. Ribosomal RNA was isolated from the ribosomes of *Ps. fluorescens* by precipitation with guanidinium chloride (Cox & Arnstein, 1962).

RESULTS

Reaction of ribosomal ribonuclease with endogenous (ribosome-bound) RNA. A comparison between the patterns of autodegradation in EDTA of untreated ribosomes from *E. coli* B (Fig. 1a) and ribonuclease-adsorbed ribosomes from *Ps. fluorescens* (Fig. 1c) suggested that these preparations had similar concentrations of EDTA-activated (Mg²⁺-independent) ribonuclease. The absence of a ribonuclease from the normal ribosomes of *Ps. fluorescens* was confirmed by the absence of RNA breakdown when the untreated ribosomes were incubated under these conditions (Fig. 1b).

The incubation of ribosomes in the presence of 1 mM-Mg²⁺ at I.O-16 revealed a major difference between the ribosomes from *E. coli* B and the ribonuclease-adsorbed ribosomes from *Ps. fluorescens*. Incubation at pH 6 for 2 hr. resulted in less than 5% degradation of the RNA in the ribosomes

Fig. 1. Autodegradation of normal ribosomes from (a) *E. coli* B and (b) *Ps. fluorescens* and of ribonuclease-adsorbed ribosomes from (c) *Ps. fluorescens*. Ribosomes (3-5 mg. dry wt./ml.) were incubated (at 37°) in 90 mM-veronal-acetate buffer for 10 min. with 20 mM-EDTA (Δ) or in 90 mM-veronal-acetate buffer for 2 hr. with 1 mM-MgCl₂ (O) or 5 mM-MgCl₂ (●) at I.O-16.

from *E. coli* B (Fig. 1a) and more than 90% in the ribonuclease-adsorbed ribosomes (Fig. 1c).

In 5 mM-Mg²⁺, conditions that maintain the ribosomes in the stable '30s' and '50s' configurations (Cammack & Wade, 1965), the RNA in the untreated ribosomes from both species was stable at pH 6. The instability of ribosomes from *E. coli* B below pH 4.5 was due to the activity of the ribonuclease, since the acid-soluble products were

hydrolysed to nucleoside 2'(3')-phosphates on treatment with alkali. A similar degradation was not observed in the untreated ribonuclease-deficient ribosomes from *Ps. fluorescens* (Fig. 1b) or *E. coli* M.R.E. 600.

In contrast with the untreated ribosomes from *E. coli* B (Fig. 1a), the ribonuclease-adsorbed ribosomes from *Ps. fluorescens* were unstable at pH 6 in the presence of 1mM- or 5mM- Mg^{2+} (Fig. 1c). Since the results also suggested that the enzyme activity is partly inhibited by the higher concentration of Mg^{2+} , the effects of varying the concentration of Mg^{2+} on the stability of ribosomal RNA at pH 6 was examined in more detail.

The results (Fig. 2) confirmed the contrasting stabilities of the two ribosome preparations. The RNA in ribosomes from *E. coli* B was stable in 2mM- Mg^{2+} , but there were signs of instability in 1mM- Mg^{2+} after incubation for 2hr. In contrast, the RNA in ribonuclease-adsorbed ribosomes from *Ps. fluorescens* was degraded at all Mg^{2+} concentrations (Fig. 2). Even in 1mM- Mg^{2+} , however, the rate of breakdown did not approach the rate in EDTA (Fig. 1c).

These results suggested that the accessibilities of enzyme to substrate were different in the ribosomes from *E. coli* B on the one hand and the ribonuclease-adsorbed ribosomes from *Ps. fluorescens* on the other, and that the normal enzyme in ribosomes from *E. coli* B did not arise by adsorption.

In an effort to strengthen this evidence, ribosomal ribonuclease from *E. coli* B was adsorbed on to an equivalent concentration of ribosomes from this species and the properties of these ribosomes

were compared with those of the normal ribosomes. The enzyme was more completely adsorbed by the ribosomes from this species than by ribosomes from *Ps. fluorescens*. About 98% was adsorbed and incubation in 20mM-EDTA confirmed the uptake of a concentration of enzyme similar to the one already present (Fig. 3a). Incubation in the presence of 1mM- Mg^{2+} showed enhanced phosphodiesterase activity above pH 7 in the ribonuclease-adsorbed ribosomes (Fig. 3b), but no general instability at pH 6 as observed with ribonuclease-adsorbed ribosomes from *Ps. fluorescens* (Fig. 1c).

The adsorbed ribonuclease was clearly reacting differently with the endogenous RNA in the two ribosome preparations, suggesting that either the RNA in the ribosomes from *E. coli* B was more effectively protected against exogenous ribonuclease or these ribosomes were inhibiting the adsorbed enzyme. To distinguish between these possibilities, the ribosomes were re-examined with exogenous RNA as a substrate for the enzyme.

Reaction of ribosomal ribonuclease with exogenous RNA. The ribonuclease activities in different Mg^{2+}

Fig. 2. Effect of Mg^{2+} concentration on the reaction of endogenous RNA with normal ribonuclease in ribosomes from *E. coli* B (●) and ribonuclease adsorbed on to ribosomes from *Ps. fluorescens* (Δ). The ribosomes (3mg./ml.) were incubated (at 37°) in 90mM-veronal-acetate buffer, pH 6, with $MgCl_2$ (1–10mM) and NaCl (10–16).

Fig. 3. Autodegradation of normal ribosomes (●) and ribonuclease-adsorbed ribosomes (○) from *E. coli* B. The ribosomes (3–5mg. dry wt./ml.) were incubated (at 37°) in 90mM-veronal-acetate buffer with (a) 20mM-EDTA for 5min. or with (b) 1mM- Mg^{2+} in 90mM-veronal-acetate buffer for 2hr.

concentrations were determined as in the previous experiment (Fig. 2) but with one-tenth the ribosome concentration.

A similar distinction was observed between the untreated ribosomes from *E. coli* B and the ribonuclease-adsorbed ribosomes from *Ps. fluorescens* (Fig. 4a) to that observed above (Fig. 2). The major difference between the two experiments was the greater activity of the ribonuclease in the untreated ribosomes from *E. coli* B between 1mm- and 5mm- Mg^{2+} towards the exogenous RNA (Fig. 4a). In 10mm- Mg^{2+} , at which concentration the effects of Mg^{2+} binding to the exogenous RNA can be ignored, the ribonuclease in the untreated ribosomes was as inactive towards the exogenous RNA (Fig. 4a) as it was towards the endogenous (ribosome-bound) RNA (Fig. 2), suggesting that an inaccessibility of the RNA in ribosomes from *E. coli* B was not responsible for the observed dissimilarity with the ribonuclease-adsorbed ribosomes from *Ps. fluorescens* (Fig. 2).

The close resemblance between the ribonuclease activities of the untreated and ribonuclease-adsorbed ribosomes from *E. coli* B (Fig. 4b) suggested instead that the suppression of activity in 10mm- Mg^{2+} (Figs. 2 and 4a) was due to an inhibition of the enzyme. The failure to distinguish between the activities of the endogenous enzyme and the adsorbed enzyme is consistent with the view that the ribonuclease in untreated ribosomes had arisen by adsorption.

When the order of mixing the reagents for this experiment was changed so that the untreated ribosomes from *E. coli* B were exposed to the RNA substrate for 2min. at 0° before the addition of the remaining reagents, little inhibition was observed in 10mm- Mg^{2+} (Fig. 4b). This residual inhibition was probably due to the weak natural inhibition that this ion exerts on the pure enzyme (Spahr & Hollingworth, 1961). In view of the dependence of ribosomes on Mg^{2+} for stability and the well-known capacity of nucleic acids to bind these ions, this result suggested that the integrity of the ribosomes is a necessary requirement for inhibition.

Inhibition of ribonuclease by ribosomes. The effect of ribosome preparations from other species of bacteria on the activity of ribosomal ribonuclease was examined to determine whether or not the inhibition displayed by ribosomes from *E. coli* B in the presence of 10mm- Mg^{2+} is exceptional. Freeze-dried preparations of ribosomes that had been prepared by differential centrifugation and dialysis against 0.1mm-magnesium sulphate (Wade & Robinson, 1965) were used.

The results showed that Mg^{2+} (10mm)-dependent inhibition of ribosomal ribonuclease from *E. coli* B is displayed by ribosomes from *A. aerogenes*, *Pr. vulgaris* and three strains of *E. coli*, but not by

Fig. 4. Effect of Mg^{2+} concentration on the ribonuclease activities of (a) the normal ribosomes from *E. coli* B (●) and ribonuclease-adsorbed ribosomes from *Ps. fluorescens* (△), and (b) the ribonuclease-adsorbed ribosomes from *E. coli* B (○), natural ribosomes from *E. coli* B and natural ribosomes from *E. coli* B that were exposed (2.5 mg./ml.) to RNA (17 mg./ml.) for 2 min. at 0° before the addition of the other reagents and incubation at 37° (▲). The ribosomes (0.3 mg. dry wt./ml.) were incubated in a solution (10.16) of 90mm-veronal-acetate buffer, pH6, containing $MgCl_2$ (1–10mm) and RNA (2mg./ml.).

ribosomes from three species of *Pseudomonas* (Table 1, columns 1–3). The apparent stimulation of activity by ribosomes from *Ps. chlororaphis* in 10mm- Mg^{2+} (Table 1, column 3) was due to the exceptional instability of these ribosomes and the consequential activation of the endogenous ribonuclease (Wade & Robinson, 1965). In view of the even greater instability of ribosomes from *Pr. vulgaris* (Wade & Robinson, 1965), it is likely that the true inhibition of the added ribonuclease by ribosomes from this species is considerably greater than the recorded value suggests (Table 1, column

Table 1. *Effect of bacterial ribosomes on ribonuclease activity*

Each type of ribonuclease (about 1–1.5 ribonuclease units/ml.) was incubated at 37° with RNA (3mg./ml.) in the presence or absence of freeze-dried ribosomes (0.5mg./ml.) in 90mm-veronal-acetate buffer, pH6, containing MgCl₂ (1 or 10mm) and NaCl (to 1.0–1.6). Strains were taken from the National Collection of Dairy Organisms (N.C.D.O.), the National Collection of Industrial Bacteria (N.C.I.B.), the National Collection of Type Cultures (N.C.T.C.) and a collection held at the Microbiological Research Establishment (M.R.E.).

Ribosomes	Natural complement of ribonuclease	Ribonuclease activity (m-moles of nucleotides liberated/l./hr.)								
		Ribonuclease from <i>E. coli</i> B			Ribonuclease from <i>Ps. chlororaphis</i>			Ribonuclease from bovine pancreas		
		1mm-Mg ²⁺	10mm-Mg ²⁺		1mm-Mg ²⁺	10mm-Mg ²⁺		1mm-Mg ²⁺	10mm-Mg ²⁺	
		(1)	(2)	(3) (% of control)	(4)	(5)	(6) (% of control)	(7)	(8)	(9) (% of control)
None (control)		1.1	0.88		1.1	0.90		1.5	1.5	
<i>Ps. chlororaphis</i> N.C.I.B. 9402	+	1.39	1.1	125	1.41	1.00	111	0.95	0.50	33
<i>Ps. fluorescens</i> N.C.I.B. 8248	—	1.1	0.89	101	1.24	0.95	105	0.50	0.16	10
<i>Ps. synchyanea</i> N.C.D.O. 759	—	1.1	0.81	92	1.12	0.99	109	1.05	0.35	23
<i>A. aerogenes</i> N.C.T.C. 418	+	3.2	0.23	26.2	3.25	1.03	114	3.0	1.4	93
<i>E. coli</i> B	+	2.7	0.23	26.2	2.55	0.90	100	3.0	0.92	61
<i>E. coli</i> M.R.E. 161	+	1.9	0.084	9.5	1.52	0.83	92	1.9	0.4	26.7
<i>E. coli</i> M.R.E. 600	—	0.48	0.021	2.4	1.12	0.90	100	1.05	0.4	26.7
<i>Pr. vulgaris</i> M.R.E. 402	+	1.3	0.55	62	1.8	1.35	150	2.7	1.5	100

3). A similar explanation can account for the excessive ribonuclease activity observed when the effects of these ribosomes on ribosomal ribonuclease from *Ps. chlororaphis* were examined (Table 1, column 6). This enzyme was not inhibited by any of the ribosome preparations (Table 1).

Since these results, and the result in Fig. 4(a), appeared to contradict an earlier experiment in which some resistance of the RNA in ribosomes from *Ps. fluorescens* to pancreatic ribonuclease was demonstrated (Cammack & Wade, 1965), the effect of ribosomes on the activity of pancreatic ribonuclease was also examined. The results (Table 1, columns 7–9) confirmed the earlier experiment and showed that pancreatic ribonuclease exerts a different pattern of inhibition from that observed with the ribosomal ribonuclease from *E. coli* B.

In view of the greater affinity of ribosomes from *E. coli* B for ribosomal ribonuclease compared with that of ribosomes from *Ps. fluorescens*, observed when the ribosomes from these species were suspended in ribosomal ribonuclease from *E. coli* B, it seemed likely that the inhibition of the ribosomal ribonuclease was the outcome of its adsorption on to the ribosome surface. This possibility was examined by studying the effect of enzyme concentration on inhibition. The activities of ribosomal ribonuclease, acting on exogenous RNA,

Fig. 5. Effect of enzyme concentration on the inhibition of ribosomal ribonuclease from *E. coli* B (●) and *Ps. fluorescens* (○). Ribosomes (0.3mg. dry wt./ml.) were incubated (at 37°) in a solution containing ribosomal ribonuclease (2.5–25 ribonuclease units/ml.) with RNA (3mg./ml.) in 90mm-veronal-acetate buffer, pH6, containing Mg²⁺ (10mm) and NaCl (1.0–1.6).

were examined in the presence of ribosomes from *E. coli* B and *Ps. fluorescens*.

A plot of the enzyme activity against enzyme concentration with ribosomes from *E. coli* B (Fig. 5) showed that, as the concentration of enzyme was increased, the initial low enzyme activity accelerated to a point at which it increased uniformly at the same rate as that observed with ribosomes from *Ps. fluorescens*. The character of the curve was consistent with an inhibition of the enzyme by its adsorption on to sites on the ribosome surface that become progressively more saturated as the concentration of enzyme is increased.

DISCUSSION

It was on the premise that ribosomes isolated from bacteria have similar properties and functions that information on the origin of the ribonuclease in ribosomes from *E. coli* B was sought from a comparison of ribosomes from two different species. The results of the first experiment (Fig. 1) showed that enzyme adsorbed on to the ribosomes from *Ps. fluorescens* was far more active towards endogenous (ribosome-bound) RNA than the native ribonuclease in ribosomes from *E. coli* B and encouraged the view that the ribonuclease in ribosomes from this species did not arise by adsorption.

The subsequent observation that ribonuclease which is adsorbed on to ribosomes from *E. coli* B is almost as inactive as the endogenous ribonuclease (Figs. 1c and 3b) confirmed a similar observation by Neu & Heppel (1964) and brought to light a major difference between the properties of ribosomes from the two species. Whereas the ribosomal RNA in ribosomes from *Ps. fluorescens* is attacked by exogenous ribonuclease under conditions of Mg^{2+} concentration (5–10 mM) and ionic strength (1.0–1.6) that maintain the ribosomes in the stable '30s' and '50s' configurations (Cammack & Wade, 1965), the RNA in ribosomes from *E. coli* B is not.

The inhibition of the enzyme by the ribosomes from *E. coli* B appears to depend on the integrity of the ribosomes. When they are subjected to low concentrations of Mg^{2+} (less than 2 mM at 1.0–1.6), conditions under which they undergo irreversible changes into more slowly sedimenting ribonucleoproteins (Cammack & Wade, 1965), inhibition becomes less effective (Figs. 2 and 4). The dependence of inhibition on a high concentration of Mg^{2+} (10 mM) appears therefore to be more directly a dependence on ribosomes in the stable '30s' and '50s' configurations. That the adsorption of the enzyme is ultimately responsible for inhibition is suggested by the relationship between enzyme concentration and inhibition (Fig. 5).

The higher ribonuclease activity of ribosomes from *E. coli* B in 1–5 mM- Mg^{2+} towards exogenous

RNA (Fig. 4a) compared with the endogenous (ribosome-bound) RNA (Fig. 2) could be due to a decrease in the effective concentration of Mg^{2+} , brought about by the added RNA, resulting in the release and activation of the enzyme. Experiments in which unlabelled ribosomes have degraded ^{32}P -labelled RNA suggest, however, that exogenous RNA is in fact more readily attacked (Neu & Heppel, 1965).

Whether or not the ribosomes from a bacterial species inhibit ribosomal ribonuclease from *E. coli* B appears to depend more on family affiliations than the possession (or otherwise) of a ribonuclease (Table 1, column 3). The results suggest that the ability of the ribosomes to inhibit this enzyme is a constitutional character rather than an adaptive response by the bacterium to protect its RNA from its own ribonuclease.

Most species of *Pseudomonas* do not have a detectable ribonuclease (Wade & Robinson, 1963, and unpublished work); *Ps. chlororaphis* is an exception. None of the ribosomes from the three species examined inhibited ribosomal ribonuclease from *E. coli* B (Table 1, column 3). The presence of a ribonuclease in *Ps. chlororaphis* and the inability of its ribosomes to inhibit the enzyme could therefore account for the instability of these ribosomes (Wade & Robinson, 1965). The situation appears to be aggravated by the exceptional potency of the ribonuclease in this species (Table 1, columns 4–6). In view of the dependence of inhibition on the integrity of the ribosomes, it could be argued that some other agent in these ribosomes is responsible for their degradation and that it is as a consequence of this that inhibition is not observed in this species. The ribosomes from *Pr. vulgaris* M.R.E. 402, however, are more unstable than those from *Ps. chlororaphis* (Wade & Robinson, 1965), and yet a significant inhibition of ribonuclease can still be detected in these ribosomes (Table 1, column 3). Considered in the light of the high ribonuclease activity observed in the presence of ribonuclease from *Ps. chlororaphis* (Table 1, column 6), it is likely that the inhibition of ribonuclease from *E. coli* B by ribosomes from *Pr. vulgaris* (Table 1, column 3) is potentially more efficient than is suggested by the value 62%.

The results provide no evidence against the origin of the ribosomal ribonuclease by adsorption. They do offer, however, an alternative explanation for the observed loss of ribonuclease from ribosomes when the concentration of Mg^{2+} (relative to the univalent cations) is decreased (Spirin, Kisselev, Shakulov & Bogdanov, 1963; Neu & Heppel, 1964, 1965) and for its exclusive location in the '30s' component (Elson, 1959; Spahr & Hollingworth, 1961; Tal & Elson, 1963; Neu & Heppel, 1964).

The irreversible changes that bacterial ribosomes

undergo when the concentration of Mg^{2+} is decreased to less than 5 mM (Cammack & Wade, 1965) is accompanied by an increase in activity of the ribosomal ribonuclease (Figs. 2 and 4), which probably reflects a lessening ability of the ribosomes to bind the enzyme. Since the initial ribosome products have sedimentation velocities not far removed from those of the stable '30s' and '50s' forms (Cammack & Wade, 1965), these changes may not easily be recognized.

It is possible that the absence of the enzyme from the '50s' component, which is currently accepted, is due to the specific conversion of this component into a more slowly sedimenting form (Cammack & Wade, 1965) when the concentration of Mg^{2+} in 10 mM-tris-hydrochloric acid buffer is decreased from 1 mM to 0.1 mM for the purpose of obtaining the '30s' and '50s' components (Tissières, Watson, Schlessinger & Hollingworth, 1959), a conversion that is obtained in sodium chloride solution (1.0–1.6) by decreasing the concentration of Mg^{2+} from 5 mM to 2.5 mM (Cammack & Wade, 1965, in Fig. 3). The '30s' component undergoes a less marked change under these conditions and perhaps retains its capacity to bind and inhibit ribosomal ribonuclease.

Unlike the ribonuclease-containing ribosomes from *E. coli* B, the ribonuclease-deficient ribosomes from *Ps. fluorescens* and *E. coli* M.R.E. 600 do not become degraded beyond more slowly sedimenting ribonucleoproteins when the concentrations of Mg^{2+} is decreased below 1 mM (Cammack & Wade, 1965). Although the sedimentation patterns of these products are similar, each showing two components with sharp sedimentation boundaries, the sedimentation velocities are significantly different in the two species (Cammack & Wade, 1965). Their contrasting abilities to inhibit ribonucleases (Table 1, columns 1 and 7) enables a further distinction to be drawn between them.

Although the quantitative aspects of the observations on ribosome inhibition may be criticized on the grounds of possible interference from other proteins in the preparation of ribosomal ribonucleases, the results in Table 1 do show a diversity of property between the ribosome preparations from different species that, like differences in RNA base sequence (Aronson, 1963; Aronson & Holowezyk, 1965) and protein composition (Waller, 1964), is hard to reconcile with a common non-specific role in protein synthesis.

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REFERENCES

- Aronson, A. I. (1963). *Biochim. biophys. Acta*, **72**, 176.
Aronson, A. I. & Holowezyk, M. A. (1965). *Biochim. biophys. Acta*, **95**, 217.
Cammack, K. A. & Wade, H. E. (1965). *Biochem. J.* **96**, 761.
Cox, R. A. & Arnstein, H. R. V. (1962). *Biochem. J.* **83**, 4 p.
Elson, D. (1959). *Biochim. biophys. Acta*, **36**, 372.
Hughes, D. E. (1951). *Brit. J. exp. Path.* **32**, 97.
Neu, M. C. & Heppel, L. A. (1964). *Proc. nat. Acad. Sci., Wash.*, **51**, 1267.
Neu, M. C. & Heppel, L. A. (1965). *J. biol. Chem.* **239**, 3893.
Roth, J. S. (1954). *J. biol. Chem.* **208**, 181.
Spahr, P. F. & Hollingworth, B. R. (1961). *J. biol. Chem.* **236**, 823.
Spirin, A. S., Kisselev, N. A., Shakulov, R. S. & Bogdanov, A. A. (1963). *Biokhimiya*, **28**, 920.
Tal, M. & Elson, D. (1963). *Biochim. biophys. Acta*, **76**, 40.
Tani, M. & Hendler, R. W. (1964). *Biochim. biophys. Acta*, **80**, 279.
Tissières, A., Watson, J. D., Schlessinger, D. & Hollingworth, B. R. (1959). *J. molec. Biol.* **1**, 221.
Wade, H. E. (1961). *Biochem. J.* **78**, 457.
Wade, H. E. & Robinson, H. K. (1963). *Nature, Lond.*, **200**, 661.
Wade, H. E. & Robinson, H. K. (1965). *Biochem. J.* **96**, 753.
Waller, J. P. (1964). *J. molec. Biol.* **10**, 319.