In Vivo Thermal Stability and Activation of *Escherichia coli* Ribosomes†

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ABSTRACT: Exposure of *Escherichia coli* MRE 600 cells suspended in standard buffer (Tris-acetate (0.01 M, pH 7.8)–$MgAc_2$ (0.01 M)–NH_4Cl (0.06 M)–β-mercaptoethanol (0.002 M)) to 53° for 15 min causes a two- to threefold increase in the poly(phenylalanine) synthesis activity of their ribosomes. Binding of [^{3}H]poly(U) and [^{3}H]Phe-tRNA by ribosomes obtained from bacteria heated at 53° was similar to that of control ribosomes obtained from bacteria heated at 37°. Peptidyltransferase activity, on the other hand, increased significantly. The degradation of poly(U) in the poly(phenylalanine) synthesis reaction mixture was lower with ribosomes obtained from heated cells as compared to control ribosomes. Therefore, the *in vivo* thermal activation of ribosomes is related both to the increase in peptidyltransferase and the decrease in nucleolytic activities. The activated ribosomes showed a normal sedimentation profile. Similar thermal treatment of the bacteria suspended in other media (for example, potassium phosphate, 0.25 M, pH 7.2) results in complete degradation of the 30S ribosomal subunit. The 50S particles which remain intact retain their peptidyltransferase activity.

It is well known that different species of bacteria are capable of growth and reproduction over a wide temperature interval, from 0° to near 100°. Each bacterial species has an optimal growth temperature below and above which development disturbances or even shock may occur. Any part of the cell (including the inner structures), and any enzyme molecule, are likely to be affected by exposure to extreme temperatures.

The effect of temperature on bacteria is extensively discussed in literature, including recent review articles (Farrell and Rose, 1967; Brown and Melling, 1971). Many of the papers deal with the thermal stability of various species of bacteria in their growth medium, the effect of different ions on this stability, and conditions for recovery of thermally shocked cells.

The effect of relatively high temperatures on ribosomes is of special importance and interest. Traub and Nomura (1969) showed that under certain ionic conditions, 40° is a critical level favorable to conformational changes permitting complete *in vitro* reassembly of the 30S particle. As was shown by Zamir *et al.* (1969) and Miskin *et al.* (1970), monovalent ion dependent reactivation of both ribosomal subunits is also pronounced at relatively high temperatures. Since the ribosomes are heterogeneous particles (at least in terms of their protein constitution, Kurland, 1970), it is quite likely that at a certain moment only part of them are active, even inside a growing cell. The question arises whether changes in the environment of the cell (ionic and temperature conditions) might induce changes in the ribosomes, resulting in increased activity.

Data presented in this article show that exposure of *Escherichia coli* cells (suspended in standard buffer) to 53° for 15 min results in a two- to threefold increase in the poly(phenylalanine) synthesis ability of the ribosomes, compared with the 37° control. Examination of poly(U) and Phe-tRNA bindings and peptidyltransferase activity showed that the significant increase in the latter in addition to the decrease in poly(U) degradation are probably the reasons for the higher ribosomal activity in poly(phenylalanine) synthesis.

Stability of the ribosomes in the thermally treated cells is closely dependent on the suspension medium. As was shown by Rosenthal and Iandolo (1970), and by Rosenthal *et al.* (1972), heating of *Staphylococcus aureus* cells in phosphate buffer causes specific degradation of the 30S particles. This observation was confirmed here and extended to other media, with the conclusion that at a certain NH_4^+ (or Na^+)/Mg^{2+} ratio as in standard buffer, heating of *E. coli* cells results in conservation of the ribosomes, on one hand, and in an increase in their specific activity in protein synthesis, on the other.

Materials and Methods

All chemicals used were of analytical grade. The water used for preparation of the ribosomes and solutions was glass distilled.

Buffers. Standard buffer: Tris-acetate (pH 7.8, 0.01 M)–NH_4Cl (0.06 M)–magnesium acetate (0.01 M)–β-mercaptoethanol (0.002 M); binding buffer: Tris-HCl (pH 7.6, 0.01 M)–KCl (0.05 M)–$MgCl_2$ (0.02 M); Tris buffer: Tris-acetate (pH 7.4, 0.001 M).

Bacteria; Growth and Thermal Treatment Conditions. E. coli MRE 600 (ribonuclease 1$^-$) (Cammack and Wade, 1965) were grown at 37° in 1% yeast extract, 2.18% K_2HPO_4, and 1.7% KH_2PO_4, and supplemented with 1% glucose. The bacteria were grown under effective aeration up to the midlog phase, harvested, and washed twice in standard buffer, 1 g wet wt/20 ml. For thermal treatment they were suspended in standard buffer (1 g wet wt/4 ml of buffer) and heated for 15 min at the desired temperature, after which the suspension was cooled in ice.

Preparation of Ribosomes. The thermally treated bacteria were broken up in a French press with cell debris removed by centrifugation for 45 min at 15,000 rpm in a Sorvall centrifuge. The crude extracts (S-30) were incubated at 37° for 30 min with DNase (Worthington), 5 μg/ml, and ATP (Sigma), 0.001 M. The ribosomes were sedimented at 105,000*g* in a Spinco preparative ultracentrifuge Model L for 3 hr. The

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supernatant (S-100) was dialyzed overnight against Tris-acetate buffer (0.01 M, pH 7.4) and frozen in small portions at −18° until use. The ribosomes were washed by two more cycles of high- and low-speed centrifugation in standard buffer, and also stored in small portions in liquid air.

Preparation of polynucleotide phosphorylase and [^3H]*poly(U)* was as described earlier (Smolarsky and Tal, 1970a).

Preparation of tRNA and [^3H]*Phe-tRNA.* These were from *E. coli* MRE 600, according to methods described by Zubay (1962).

Binding of [^3H]*Poly(U) to Ribosomes.* The reaction mixture contained, in the total volume of 0.5 ml of binding buffer, 2 A_{260nm} units of ribosomes and 0.420 A_{260nm} unit of [^{3}H]-poly(U) (20,500 cpm/A_{260nm}).

Binding was carried out as described earlier using the method of alkali-treated Millipore membranes (Smolarsky and Tal, 1970b).

Binding of [^3H]*Phe-tRNA to ribosomes* was as described by Nirenberg and Leder (1964).

Incorporation of [^3H]*Phenylalanine.* Incorporation of [^{3}H]-phenylalanine was carried out according to Nirenberg and Matthaei (1961), with modifications. The reaction mixture contained in 0.5 ml: KCl, 0.054 M; NH_4Cl, 0.02 M; $MgCl_2$, 0.018 M; Tris-acetate (pH 7.8), 0.10 M; β-mercaptoethanol, 0.002 M; ATP, 0.001 M; GTP, 0.0001 M; total *E. coli* tRNA, 3.0 A_{260nm} units; phosphocreatine, 1 mg (Sigma); creatine phosphokinase, 0.25 mg (Sigma); poly(U), 2.0 A_{260nm} units (Miles Laboratories); 6 μCi of L-[^{3}H]phenylalanine, 1 Ci/mmol (Radiochemical Centre); and 1.5 μg each of the other 19 amino acids; S-100 supernatant, 1 mg of protein obtained from unheated bacteria; ribosomes, 5 A_{260nm} units or other amounts as described in the legend.

The reaction mixture was incubated at 37° for 20 min, and polymerization was then terminated by addition of an equal volume of cold 10% trichloroacetic acid solution containing 0.2% D,L-phenylalanine, and 2.5 ml of 5% CCl_3COOH containing 0.1% D,L-phenylalanine. The tubes were transferred to a boiling-water bath for 30 min, cooled in ice, and then centrifuged at 10,000 rpm for 10 min in a cooled Sorvall centrifuge. The pellet was dissolved in 1 ml of 0.2 M KOH by shaking the tube in a Vortex, and protein was precipitated with 1 ml of cold 10% CCl_3COOH solution containing 0.2% D,L-phenylalanine, followed by 2.5 ml of 5% CCl_3COOH solution containing 0.1% D,L-phenylalanine. The suspension was filtered through a Whatman glass filter paper GF/A and washed thoroughly with five portions, 10 ml each, of the latter CCl_3COOH solution. The wet filters were soaked with 0.1 ml of 0.2 M KOH to dissolve the flocculated protein and dried under an infrared lamp. The dried filters were transferred to 10 ml of scintillation liquid containing 4 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)] benzene per l. of toluene and counted in a Packard Tri-Carb scintillation spectrometer.

The experimental values given are after subtraction of the "zero time" of incubation which was ~1500 cpm.

Assay of Peptidyltransferase Activity by "Fragment Reaction." Peptidyltransferase was assayed by the "fragment reaction" developed by Monro and Marcker (1967) and modified by Miskin *et al.* (1970). Each reaction mixture was in a volume of 100 μl and contained 60 mM Tris-HCl (pH 7.4), 400 mM KCl, 20 mM $Mg(Ac)_2$, 1 mM neutralized puromycin (Nutritional Biochemical Corp., Cleveland, Ohio), 1 A_{260nm} unit of ^{14}C-labeled fMet-tRNA (218 Ci/mol) (kindly donated by Drs. N. Sonenberg, R. Miskin, A. Zamir, and D. Elson), and ribosomes as indicated in the legend. The reaction was initiated by addition of 25 μl of methanol, carried out at 0° for 15 min. The radioactive reaction product was measured according to Kinard (1957).

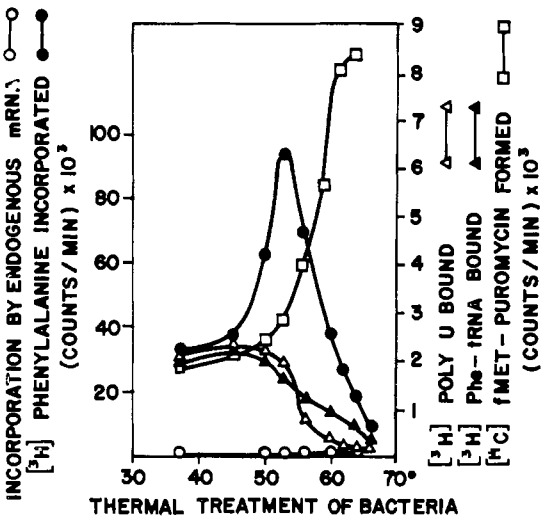

FIGURE 1: Activity of ribosomes from bacteria heated to different temperatures in standard buffer. Thermal treatment and preparation of ribosomes were as described under Materials and Methods. Amount of ribosomes—for [^{3}H]poly(U), for [^{3}H]Phe-tRNA binding, and for peptidyltransferase assay, 2 A_{260nm} units; for poly-(phenylalanine) synthesis, 5 A_{260nm} units; for endogeneous mRNA-directed protein synthesis, 10 A_{260nm} units. For the latter assay, ten tritium-labeled amino acids (~3 nmol each, total radioactivity 660,000 cpm) were used: L-[^{3}H]lysine (197 Ci/mol), DL-[^{3}H]serine (126 Ci/mol), L-[^{3}H]phenylalanine (7.45 Ci/mol), L-[^{3}H]methionine (5.4 Ci/mmol), L-[^{3}H]proline (266 Ci/mol), L-[^{3}H]arginine (151 Ci/mol), DL-[^{3}H]glutamic acid (39 Ci/mol), L-[^{3}H]histidine (40 Ci/mol), [^{3}H]glycine (2.33 Ci/mol), and L-[^{3}H]alanine (100 Ci/mol). The mixture was supplemented with the other ten amino acids, 1.5 μg each. Procedures for [^{3}H]poly(U), [^{3}H]Phe-tRNA binding, peptidyltransferase assay, and protein synthesis were as described under Materials and Methods.

Spectrophotometry. A Zeiss spectrophotometer Model M_4 QIII was used for absorbance measurements. Ribosome and protein concentrations were determined as described earlier (Tal, 1969).

Analytical Ultracentrifugation. A Beckman Model E analytical ultracentrifuge equipped with electronic speed control and a photoelectric scanner for ultraviolet optics was used for determination of the sedimentation constants.

Results

Figure 1 shows that ribosomes obtained from preheated resting cells of *E. coli* MRE 600, harvested at their midlog phase, exhibited various levels of activity (in terms of poly-(phenylalanine) synthesis) compared with the control (ribosomes from unheated cells); 53° is the optimal temperature for obtaining the highest ribosomal activity—about double that of the control; higher preheating temperatures cause a very steep drop in activity. No increase of [^{3}H]poly(U) binding ability of the ribosomes was observed at this optimal temperature, and a reduction occurred at higher temperatures. Binding of [^{3}H]Phe-tRNA to the various ribosomal preparations was similar to poly(U) binding. The probability that the increased activity in poly(phenylalanine) synthesis was due to the absence of endogenous mRNA was ruled out. As is shown in Figure 1, none of the ribosomal preparations contain endogenous mRNA: neither the 37° variant, nor the 53° one.

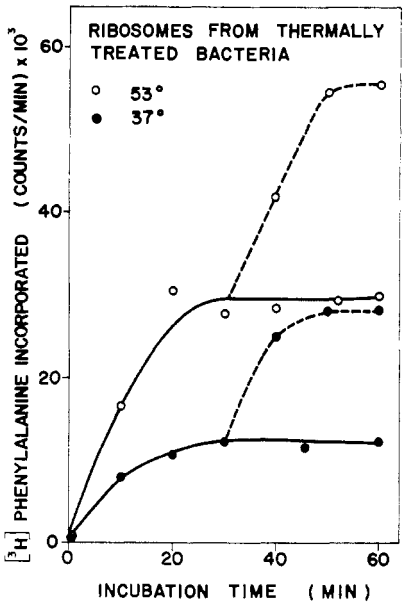

FIGURE 2: Kinetics of poly(phenylalanine) synthesis by ribosomes obtained from heated bacteria. Bacterial cells were heated in standard buffer at 37 or 53° for 15 min. Preparation of ribosomes and poly(phenylalanine) synthesis assay were as described under Materials and Methods. In accordance with the aliquots taken from the reaction mixture, each experimental value represents the reaction product of 2 A_{260nm} units of ribosomes. The solid line represents poly(phenylalanine) synthesis. After 30-min incubation another portion of poly(U) was added. The dashed line represents the enhanced poly(phenylalanine) synthesis. Ribosomes from bacteria heated at 37° (●); ribosomes from bacteria heated at 53° (○).

The possibility that the pattern of the various ribosomal preparations in poly(phenylalanine) synthesis activity is due to a monovalent ion dependent activation process similar to Elson's was also examined. Since all our ribosomal preparations were purified in standard buffer (containing NH_4^+ ions), *inactivation* during isolation was unlikely. Indeed we observed that exposure of the ribosomal preparations to activation

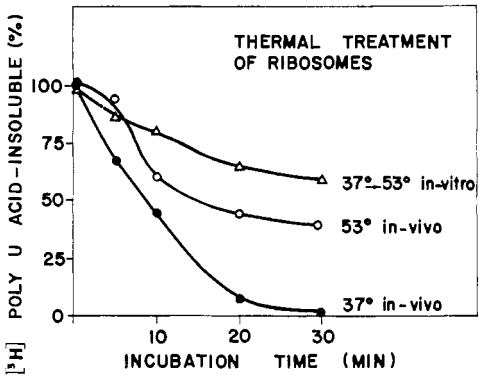

FIGURE 3: Ribosome-associated [³H]poly(U) nucleolytic activity. The reaction mixture in a 1-ml volume contained 2 A_{260nm} units of ribosomes, 6 A_{260nm} units of [³H]poly(U) (specific radioactivity 20,500 cpm/A_{260nm}), Tris buffer, salts, ATP, GTP, tRNA, amino acids, creatine kinase, and phosphocreatine as in the poly(phenylalanine) synthesis system: (●) ribosomes obtained from 37° treated cells; (○) ribosomes obtained from 53° treated cells; (△) ribosomes obtained from 37° treated cells and heated at 53° for 10 min in standard buffer. The incubation was carried out at 37°. Aliquots of 0.2 ml were taken, mixed with cold 5% trichloroacetic acid, and filtered through Millipore membrane filters. The filters were washed with three portions of 5% CCl_3COOH (10 ml each), and dried under an infrared lamp. Radioactivity was counted in the Tri-Carb spectrometer.

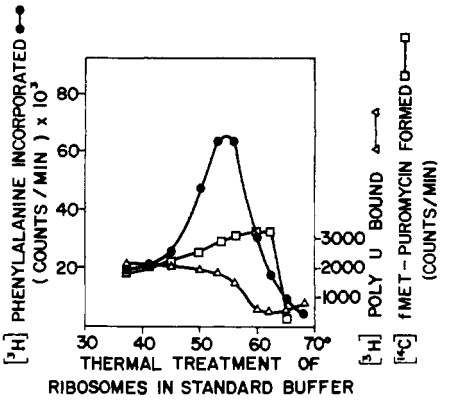

FIGURE 4: Activity of ribosomes after heating at different temperatures. Ribosomes extracted from *unheated* bacteria were dissolved in standard buffer in a concentration of 82 A_{260nm}/ml. The ribosomal solutions were exposed to elevated temperatures indicated for 10 min and cooled in ice. Procedures for [³H]poly(U) binding, poly(phenylalanine) synthesis, and peptidyltransferase activity were as described under Materials and Methods.

conditions (Miskin *et al.*, 1970; Zamir *et al.*, 1971) (NH_4Cl (0.5 M)–$MgCl_2$ (0.01 M)–2-mercaptoethanol (6 mM) at 37°) for 0, 20, 45, and 90 min did not result in increase of activity of any ribosomal preparation. Therefore, this *in vitro* reactivation step was omitted from the series except for the experiment described later in Table II.

Examination of the different ribosomal preparations for their peptidyltransferase activity showed a remarkable increase up to the highest temperature of pretreatment of bacteria—66°, as is also seen in Figure 1. The possibility that the increase in peptidyltransferase activity is due to progressive depletion of peptidyl-tRNA from the ribosomes caused by the thermal treatment of the bacteria was ruled out on the following grounds. Ribosomal preparations which underwent a wash in 1 M NH_4Cl during the purification process showed the same peptidyltransferase and poly(phenylalanine) synthesis patterns, identical with the data given in Figure 1. Moreover, the activity of ribosomes treated with puromycin in a procedure based on that of Haenni and Lucas-Lenard (1968)[1] was found to be identical with the control.

The kinetics of [³H]phenylalanine incorporation by ribosomes obtained from the heated and control cells is shown in Figure 2. The ribosomes from heated bacteria (53°) have a higher initial rate as well as a higher level of poly(phenylalanine) synthesis. After the plateau was reached, another amount of poly was added, resulting in a similar response in terms of the amount of poly(phenylalanine) synthesized. The ribosome-associated nucleolytic activity is shown in Figure 3.

In another series of experiments, ribosomes obtained from *unheated* cells were exposed to elevated temperatures followed by [³H]poly(U) binding, [³H]phenylalanine incorporation, and peptidyltransferase activity assays. Results (Figure 4) indicate that the ribosomes dissolved in standard buffer exhibit a thermal activation pattern similar to that obtained after heating the whole cell, except for the peptidyltransferase

[1] 50 mM Tris-HCl, pH 7.4, 10 mM $MgAc_2$, 160 mM NH_4Cl, 10 mM 2-mercaptoethanol, 0.2 mM GTP, 1 mg of protein S-100 fraction, 100 A_{260nm} units of ribosomes, and 1 mM puromycin in a total volume of 1 ml were incubated at 37° for 15 min. The reaction mixture was layered in a Spinco ultracentrifuge tube on 5 ml of 5% glycerol layered on 3 ml of 20% glycerol all in standard buffer. The ribosomes were sedimented for 4 hr in 50,000 rpm at 4°. The ribosomal pellets were dissolved in standard buffer and peptidyltransferase activity was measured as described under Material and Methods.

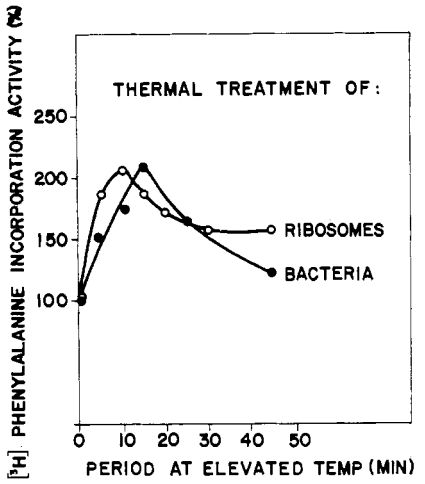

FIGURE 5: Duration of thermal treatment and its effect on ribosomal activity. Bacteria were suspended in standard buffer and ribosomes were obtained from unheated bacteria also dissolved in standard buffer; both were heated at 53° for periods indicated. Preparation of ribosomes and procedure for poly(phenylalanine) synthesis were as described under Materials and Methods.

TABLE I: Incorporation of [³H]Phenylalanine in Pure Ribosomes Obtained from Bacteria Suspended and Heated in Different Media.[a]

Heating Medium	[³H]Phe Incorpd (cpm) by Ribosomes Obtained from Bacteria Heated to 37°	53°
1. Standard buffer	22,891	59,710
2. 0.5 M NH_4Cl + 0.01 M $MgCl_2$	19,706	30,651
3. 0.5 M NaCl + 0.01 M $MgCl_2$	18,734	898
4. Growth medium (glucose eliminated)	20,796	1,760
5. 0.25 M, pH 7.2 potassium phosphate	17,814	1,582
6. 0.25 M sucrose	23,903	6,609
7. Standard buffer, Na^+ replacing NH_4^+		56,318

[a] Bacteria suspended and heated at 37 or 53° for 15 min in indicated media. Washing of heated bacteria, ribosomal preparation, and procedure for poly(phenylalanine) synthesis as described in legend to Figure 7 and under Materials and Methods.

activity, which in the *in vitro* thermal treatment is not stable above 60°, similar to the *in vitro* thermal inactivation described by Kikuchi and Monier (1971). This observation conflicts with the *in vivo* thermal treatment which activates this enzyme progressively up to 66°.

The kinetics of the thermal treatment, at 53°, of intact bacteria as well as of ribosomes dissolved in standard buffer is illustrated in Figure 5. The two curves are comparable, the time for maximal activation on exposure to 53° being 15 min for the intact cells and 10 min for the *in vitro* variant.

The biological effect of the thermal treatment (as described under Materials and Methods) on the intact cells was examined in terms of their viability and growth rate. The viability was found to remain constant up to 50°, beyond which the cells undergo a steep decline with eventual loss of their ability to divide at 60°. The growth curves of bacteria preheated to 50, 53, and 56° were examined. All curves are similar both in growth rate and in the bacterial density in the stationary phase, except for the progressive lag periods observed: 0.5, 1.5, and 9.0 hr, respectively, *vs.* the 37° control—the only explanation for this being reduction in the number of viable cells, combined with a probable metabolic injury. In fact, a considerable proportion of the colonies in the 53 and 56° variants were significantly small (about 1-mm diameter, as against the normal size of 3 mm). After another 24 hr, however, the small colonies began to recover and eventually reached normal size.

Figure 6 shows sedimentation profiles of preparations obtained from bacteria heated in standard buffer: the crude extract (S-30), ribosomes after purification in standard buffer (70 S) and ribosomal subunits obtained after dialysis of the 70S particles against Tris (1 mM, pH 7.4; 30 S + 50 S). In the S-30 group, the absorbance curves show that the higher the temperature to which the cells were exposed, the lower the relative amount of ribosomes: ~60% of 260-nm-absorbing material at 37° as against ~20% in the 66° variant. As regards the sedimentation coefficients, the 70S particle appears throughout (with values from 64 to 69 S), the 50S subunit, from 60° upward, its relative amount increasing with exposure temperature. This appearance of the 50S particles is not accompanied by any parallel appearance of the 30S subunit, undoubtedly destroyed at the elevated temperature. The proportions of the 70S and 50S components on the one hand, and the absence of 30S subparticle on the other, are clearly seen in the 70S section; the decrease of the 30S particle is again apparent in the 30S + 50S section.

The effect of heating the intact cells at 53° for 15 min in six different media is shown in Figure 7. Heating in standard buffer with NH_4^+ replaced by Na^+, at the same concentration, resulted in the same sedimentation profiles for the S-30 and 70S section as in the regular standard buffer. With three of the media (*sodium chloride*, *growth medium*, and *potassium phosphate*), the S-30 preparations, notably, showed complete absence of the 30S particles, so that the ribosomes in all three sections are seen to comprise the 50S subunit only. With ammonium chloride (0.5 M) + $MgCl_2$ (0.01 M), the thermal stability of the 70S particles was relatively high, although less than in the case of the standard buffers. With *sucrose*, almost complete degradation of the 30S particles was again observed, with the result that a minute amount of 70S particles was detected in the crude extract and in the pure ribosomal section, and very small amounts of free 30S particles in the "dissociated subunits" section. It should be noted that the sedimentation profiles of the controls (37°) for all media were alike and normal in terms of the relative amount of 70S particles in the crude extracts. It should be emphasized that after the treatment in any of the above media, the bacteria were washed in standard buffer prior to their disruption.

The poly(phenylalanine) synthesis activity of the various preparations, compared with their respective controls, is given in Table I, which shows a correlation between the activity and the amount of 70S particles in the preparations.

The intrinsic activity of the 50S particles was examined by measuring their peptidyltransferase activity (Table II). It is seen that the 50S particles obtained from bacteria suspended and heated in 0.25 M potassium phosphate buffer (pH 7.2) or in 0.5 M NaCl + 0.01 M $MgCl_2$, at 53° for 15 min (expt 3 and 4), retained significant activity compared with their control counterparts (expt 1 and 2). In an experiment not described here, it was found that these 50S particles are also active in

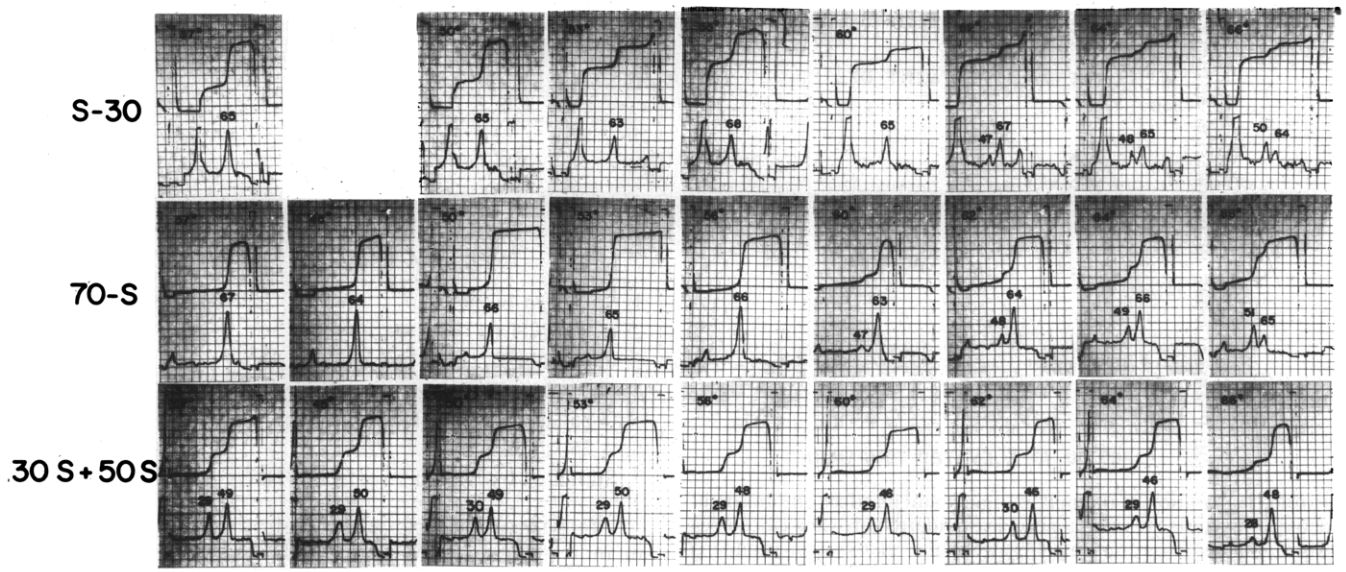

FIGURE 6: Sedimentation profiles of ribosomes obtained from bacteria heated in standard buffer. Preparation of fractions was as described under Materials and Methods. Crude extract (S-30) and purified ribosomes (70 S) were dissolved in standard buffer and dissociated-subunits fraction (30 + 50 S) were obtained after dialysis against 1 mM Tris buffer. Concentration of solutions was analyzed for sedimentation pattern 1 A_{260nm}/ml. Plots were recorded on analytical ultracentrifuge: upper absorbance at 260 nm, lower derivative with sedimentation constants given as $s_{20,w}$ (S).

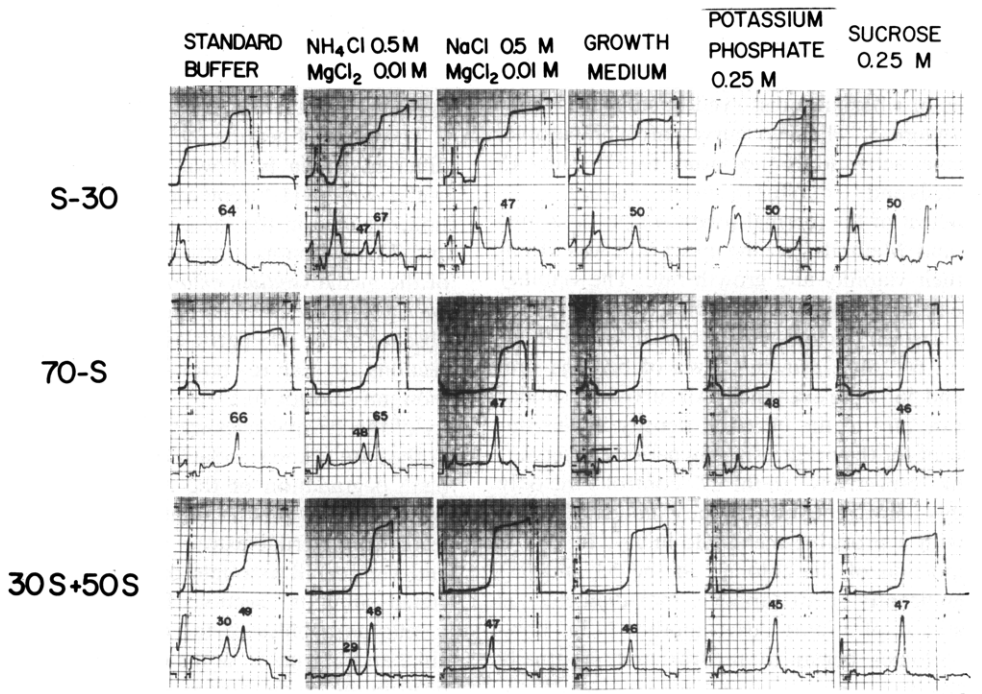

FIGURE 7: Sedimentation profiles of ribosomes obtained from bacteria heated in different media. Washing of bacteria was as described under Materials and Methods, except that different media, as indicated, were used. Bacterial suspensions were heated at 53° for 15 min. Cells sedimented in Sorvall, and were washed twice in standard buffer and suspended in the same buffer. Preparation of fractions was as described under Materials and Methods. S-30 and 70S fractions were dissolved in standard buffer; 30S + 50S fractions were obtained after dialysis against 1 mM Tris buffer. For analytical ultracentrifugation, concentration in all cases was 1 A_{260nm}/ml: upper plot, absorbance at 260 nm; lower plot, derivative with sedimentation constants given as $s_{20,w}$ (S).

poly(phenylalanine) synthesis with supplemented 30S particles.

Degradation of the 30S subunit during heating of the bacterial cell at 53° in 0.25 M phosphate buffer (pH 7.2) was studied kinetically, by examining crude extracts in the analytical ultracentrifuge and determining the amount of each particle from the absorbance curves. As is seen from Figure 8, at zero time there are only 70S particles, which comprise 50% of the material absorbing at 260 nm. After 15-min heating, none of the 70S particles remain. During degradation of the 70 S, a small amount of 30 S can be detected, but in view of their thermal instability none of them remain after the 15-min period. The increase in the amount of 50S particles is clearly associated with the disappearance of the 70S ribosomes.

TABLE II: Peptidyltransferase Activity in Isolated 50S Particles from Bacteria Heated in Different Media.[a]

Expt No.	Heating Medium of the Bacteria	Temp (°C) of Thermal Treatment	[^{14}C]fMet-Puromycin Formed (cpm)
1	Standard buffer	37	2502
2	Standard buffer	53	4261
3	0.25 M, pH 7.2 potassium phosphate	53	3053
4	0.5 M NaCl + 0.01 M $MgCl_2$	53	2461

[a] Ribosomes obtained from bacteria heated in above media as described in legend to Figure 7 and under Materials and Methods. Ribosomes dissociated into subunits by overnight dialysis against 1 mM Tris-acetate (pH 7.4) containing 10^{-4} M $MgAc_2$. 50 A_{260} units of ribosome in volume of 0.5 ml layered above 34 ml of 10–30% v/v linear glycerol gradient (in the same buffer). Centrifugation at 4° in SW 27 rotor in preparative ultracentrifuge (Spinco Model L), for 15 hr at 21,000 rpm. Fractions for analysis, collected from bottom of tube through Gilford Model 2000 spectrophotometer, and absorbance at 260 nm recorded automatically. Fractions containing 50S particles were pooled together, concentrated, and washed (using the same buffer) by means of Amicon ultrafiltration Diaflo membrane PM-30. The ribosomal purity was checked on Beckman Model E analytical ultracentrifuge. 50S particles from standard buffer not less than 95% pure, other two 100% pure. Each ribosomal preparation underwent reactivation step (Zamir *et al.*, 1969) in 0.5 M NH_4Cl containing 0.01 M $MgCl_2$ at 37° for 45 min. 0.2 A_{260nm} unit of each ribosomal preparation was analyzed for peptidyltransferase activity as described under Materials and Methods.

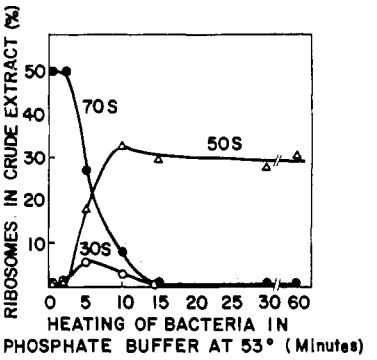

FIGURE 8: Relative content of ribosomal particles in crude extract of bacteria heated in phosphate buffer. Bacteria were washed and suspended in 0.25 M potassium phosphate buffer (pH 7.2), exposed to 53° for various periods of time. Crude extract fractions were recorded on analytical ultracentrifuge. The relative amount of each particle was calculated from its absorbance value.

Discussion

It was shown that *E. coli* cells heated in a certain ionic environment (standard buffer) undergo a very pronounced increase in the poly(phenylalanine) synthesis ability of their ribosomes. The optimal exposure temperature is 53°, and the corresponding increase is two- to threefold compared with the 37° control; at higher temperature levels of exposure, a sharp decrease in activity is observed.

This higher activity could be due to several causes. One such cause could be accelerated degradation of endogenous mRNA in the polysomes, as a result of which the ribosomes become mRNA deficient and thus more available for translating the supplied poly(U) in the Nirenberg system. Figure 1 shows that none of the endogenous mRNA is still bound—as could be expected, since the crude extracts were incubated at 37° and the ribosomes are probably of the run-off type (Kohler *et al.*, 1968). In confirmation of this notion, our experiment with puromycin-treated ribosomes should be mentioned, which have the same ability to form fMet-puromycin as their untreated counterparts. Thus our ribosomal preparations are probably not only run-off ribosomes but in addition also peptidyl-tRNA-depleted particles.

Another possible cause is inactivation of certain ribosomal preparations (obtained from the 37, 45, and 50° variants) which showed relatively low activity compared with the 53° variant. This is, however, unlikely, since all ribosomal preparations were prepared in the presence of NH_4^+ ions (Miskin *et al.*, 1968). We may thus conclude that the thermal activation described here is of a different nature.

The ribosomal preparations were examined with regard to various steps in the protein biosynthesis pathway, namely poly(U), and Phe-tRNA bindings and peptidyltransferase activity. Regarding the first two steps, no significant change was observed up to the 53° variant (and even a slight decrease at the 53°). As regards the third step, Figure 1 shows that thermal pretreatment of the bacteria causes a progressive increase in it up to 66° (the top limit of our experiments), which accordingly may contribute to the increase in poly(phenylalanine) synthesis in the 53° variant. At higher temperatures, the net result is a steep drop in poly(phenylalanine) synthesis, caused by a decrease in poly(U) and Phe-tRNA bindings. It should be borne in mind that strictly speaking, there is no accurate parallelism between the increases in peptidyltransferase and poly(phenylalanine) synthesis activities. Our experiments, described in Figures 2 and 3, showed that the degradation of poly(U) in the poly(phenylalanine) synthesis system is more pronounced in the 37° variant compared to the 53° ribosomes. Indeed, the lower poly(U) degradation activity associated with ribosomes from heated bacteria affects the translating efficiency in the same direction as the activation of peptidyltransferase in these particles. In analogy to the *in vivo* ribosomal thermal activation, the *in vitro* thermal treatment caused similar activation of poly(phenylalanine) synthesis ability (Figure 4) accompanied by inactivation of ribosomal nucleolytic activity (Figure 3). Thus, the combined result is the ribosomal thermal activation described here.

We should also take into consideration the possibility that the thermal treatment of the bacteria causes a release of membrane bound ribosomes. If such ribosomes either have a higher activity of peptidyltransferase or this enzyme is more readily activated as compared to the enzyme in the cytoplasmic ribosomes, we may have observed this *in vivo* thermal activation of ribosomes. On the other hand, the *in vitro* thermally treated ribosomes (Figure 4) show a similar activation in poly(phenylalanine) synthesis which may be attributed both to the *activation* of peptidyltransferase and to the *inactivation* of ribosomal bound nucleases, with one difference regarding the instability of peptidyltransferase at relatively high temperatures which might be due to the differences between the *in vivo* and *in vitro* environmental conditions. Therefore, we believe that the

cytoplasmic ribosomes undergo the thermal activation in poly(phenylalanine) synthesis described here.

The thermally induced *in vivo* activation of the ribosomes may involve only small conformational changes (if any) in the ribosomes. We base it on identical melting profiles (Tal, 1969) (not presented in this article) of the *in vivo* activated (53° variant) ribosomes and the control ribosomes (37° variant); this is in addition to the identical sedimentation profiles shown in Figure 6. Nevertheless, the hypothetical conformational changes were stable throughout the whole procedure of ribosomal preparation.

From the viability and growth-curves experiment, it is clear that the heat treatment causes a thermal shock. Taking into consideration our *in vitro* analyses of the ribosomal activity (Figure 1) it may be concluded that the ribosomal system of *E. coli* cells suspended in standard buffer is of outstanding thermal stability. Therefore, any damage to enzymes involving one of the biosynthesis pathways or even the membrane may be remedied by new synthesis of proteins, which in turn may be promoted by the increased activity of the ribosomes as measured *in vitro*.

Iandolo and Ordal (1966), Sogin and Ordal (1967), Rosenthal and Iandolo (1970), and Rosenthal *et al.* (1972) studied the effect of elevated temperatures on *Staphylococcus aureus* cells. They used phosphate buffer as heating medium. The effect on the ribosomes was observed at temperatures as low as 48°. By contrast, our results show that heating of *E. coli* cells suspended in standard buffer imparts higher stability to the ribosomes and, moreover, *in vivo* activation of the ribosomes was observed.

The experiment in which *E. coli* cells were heated in different media confirmed the selective degradation of the 30S particles, as also found by Rosenthal and Iandolo (1970) and by Rosenthal *et al.* (1972). (It could very well be that the ratio of mono- to divalent ions is a factor in the thermal stability.) By contrast, the 50S particles are stable under the experimental conditions described and retain their peptidyltransferase activity (Table II). The nature of the medium in which the bacteria are suspended is of utmost importance as regards the intracellular nucleases activities, since after heating in standard buffer there is a *reduction* in ribosome associated nuclease activity, while heating in another medium, for example, potassium phosphate buffer, causes *activation* of certain nucleases which results in a complete degradation of the 30S ribosomal subunits. From our own experiments, it is clear that the 50S particles remain active after the thermal treatment. It may be concluded, therefore, that for recovery from thermal shock involving ribosomal degradation, the bacteria have to reassemble the 30S particles (Rosenthal *et al.*, 1972) and may utilize the remaining active 50S particles present.

The specific intracellular degradation of the 30S particles at elevated temperatures may be due to their associability with certain nucleases inside the bacterial cell (Tal and Elson, 1963; Natori *et al.*, 1967). Neu and Heppel (1964a) found that *E. coli* cells may undergo depletion of RNase, from which they concluded that the enzyme is not bound to the 30S particles but located close to the bacterial membrane. On the other hand, they also found (1964b) that ribosomes loaded (*in vitro*) with a large excess of RNase show increased binding of the enzyme to the 30S particle *only*. In the light of the above, and of our own results regarding the specific thermal susceptibility of the 30S particle, we conclude that certain nucleases undergo, *at least* in a transient form, specific binding to the 30S ribosomal particle in the bacterial cell.

Conclusion

Heating of *E. coli* cells in certain media resulted in complete degradation of the 30S ribosomal particle, while the 50S remained intact and retained its biological activity. In another type of medium (standard buffer), not only were the ribosomes more stable, but the thermal treatment actually resulted in increase in their poly(phenylalanine) synthesis ability. In fact, this thermal treatment unexpectedly caused very considerable activation of peptidyltransferase on the one hand, and a significant decrease in ribosome-associated nuclease activity on the other. It is self-evident that both act in the same direction and resulted in the thermal activation of ribosomes, described here.

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