

## Re-activation of the Peptidyltransferase Centre of Rabbit Reticulocyte Ribosomes after Inactivation by Exposure to Low Concentrations of Magnesium Ion

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(Received 14 June 1976)

1. The larger subribosomal particles of rabbit reticulocytes retained full activity in the puromycin reaction and in poly(U)-directed polyphenylalanine synthesis after 4 h at 0°C when buffered 0.5 M-NH<sub>4</sub>Cl/10–30 mM-MgCl<sub>2</sub> was the solvent. 2. Activity in the puromycin reaction was diminished to approx 10% after 15–30 min at 0°C when the concentration of MgCl<sub>2</sub> was lowered to 2 mM. 3. Activity was not restored when the concentration of MgCl<sub>2</sub> was raised from 2 mM to 10–30 mM at 0°C. However, activity was recovered as measured by both assay systems when the ribosome fraction was heated to 37°C at the higher concentrations of MgCl<sub>2</sub>. 4. Recovery of activity was noted during the course of the polyphenylalanine synthesis in 50 mM-KCl/5 mM-MgCl<sub>2</sub>/25 mM-Tris/HCl, pH 7.6, at 37°C. Re-activation was slow at 20°C and below. 5. No more than about 5% of the protein moiety of the subparticle was lost in 0.5 M-NH<sub>4</sub>Cl on decreasing MgCl<sub>2</sub> concentration from 10 mM to 2 mM. No proteins were detected in the supernatant fractions by gel electrophoresis after ribosomes were separated by differential centrifugation. The supernatant fraction was not essential for the recovery of activity. However, at higher (e.g. 1 M) concentrations of NH<sub>4</sub>Cl, proteins were split from the subparticle. 6. The loss and regain of activity found on lowering and restoring the concentration of MgCl<sub>2</sub> at 0.5 M-NH<sub>4</sub>Cl appears to arise from a conformational change that does not seem to be associated with a loss and regain of particular proteins. 7. A 2% decrease in  $E_{260}$  was noticed when the concentration of Mg<sup>2+</sup> was restored, and the change in the spectrum indicated a net increase of approx. 100 A·U base-pairs per subribosomal particle. 8. When the concentration of Mg<sup>2+</sup> was restored,  $s_{20,w}$  of the subparticle remained at 52 ± 1 S until the sample was incubated at 37°C when  $s_{20,w}$  increased to 56 ± 1 S compared with the value of 58 ± 1 S for the subparticle as originally isolated.

The capacity to treat subribosomal particles so that well-defined protein-depleted core particles are produced, together with a split-protein fraction comprising a few proteins, has proved to be useful in establishing structure–function relationships in *Escherichia coli* ribosomes (for a review, see Nomura, 1973). Attempts to produce core particles from eukaryotic ribosomes has met with little success (for a review, see Wool & Stöffler, 1974).

One way of depleting ribosomes of proteins is to manipulate the ionic conditions (Lerman, 1968; Clegg & Arnstein, 1970; Reboud *et al.*, 1972). Following this approach we used, as a reference point, 0.1 M-NH<sub>4</sub>Cl/2 mM-MgCl<sub>2</sub>/1 mM-dithiothreitol/0.02 M-Tris/HCl, pH 7.6, in which the larger subribosomal particle (L-subparticle) of the rabbit is stable (Falvey & Staehelin, 1970), and then increased the concentration of NH<sub>4</sub>Cl. After treatment at 0°C for 2 h with higher concentrations (e.g. 1 M) of NH<sub>4</sub>Cl the activity

of the L-subparticle in poly(U)-directed polyphenylalanine synthesis was diminished and proteins were split off. However, the aim of the present paper is to show that the L-subparticle was inactivated at an intermediate concentration (0.5 M-NH<sub>4</sub>Cl) without detectable loss of protein, and that the altered L-subparticle was re-activated by first raising the concentration of Mg<sup>2+</sup> to at least 10 mM and then heating at 37°C; and that a conformational change, as judged by spectrophotometry and by sedimentation studies, was concomitant with inactivation.

The results suggest that the tertiary structure of rRNA, as maintained by Mg<sup>2+</sup>, is important to the peptidyltransferase function of the ribosome. The need for approx 1 Mg<sup>2+</sup> ion/40 NH<sub>4</sub><sup>+</sup> ions to preserve the active conformation of the rabbit L-subparticle is in accord with an analysis of the interaction of Mg<sup>2+</sup> with rRNA [see the preceding paper (Cox & Hirst, 1976)].

## Methods

### General Methods

*U.v. spectrophotometry.* A Unicam SP. 500 or a Unicam SP. 700 spectrophotometer was used for routine measurements. A Cary model 118 spectrophotometer was used for the measurement of difference spectra; a difference in extinction of 0.01 was measured to within 1%.

*Ultracentrifugation.* MSE low-speed (Mistral and 18) and high-speed (Superspeed 40, Superspeed 50, and Superspeed 65) centrifuges were used for all preparative work. An MSE 65 ultracentrifuge fitted with a B XIV rotor was used for zonal centrifuging. A Beckman model E ultracentrifuge fitted with u.v. and schlieren optics was used for analytical work. In the u.v. optical path the camera was replaced by a photoelectric cell that scanned the slit, and the movement of the sedimenting species in a 12mm-path-length cell was followed by means of a plot of percentage transmission against distance on an *xy* recorder. All u.v. analytical studies were made at temperatures in the range 5–20°C with a ribosome concentration of about 0.05 mg/ml. When the schlieren optical system was used, the phase-plate angle was set at 60°.

### Preparation and isolation of rabbit reticulocyte ribosomes, pH5 enzyme fraction and $(\text{NH}_4)_2\text{SO}_4$ fraction

Rabbit reticulocytes were prepared from phenylhydrazine-treated rabbits and were lysed, as described by Arnstein *et al.* (1964). The polyribosome fraction and the pH5 enzyme fraction were isolated from the cell lysate as described in the preceding paper (Cox & Hirst, 1976). An  $(\text{NH}_4)_2\text{SO}_4$  fraction containing elongation factors was isolated by the procedure of Hardesty *et al.* (1971).

### Dissociation of the polyribosome fraction into subparticles, and separation by zonal centrifuging

The polyribosome fraction was dissociated by incubation at 37°C for 30 min in 0.5 M-KCl/3 mM-MgCl<sub>2</sub>/1 mM-dithiothreitol/20 mM-Tris/HCl, pH 7.6, together with 0.2 mM-GTP and 0.2 mM-puromycin, and then the subunits were separated by zonal centrifugation in a 15–45% (w/v) sucrose gradient containing 0.3 M-KCl/3 mM-MgCl<sub>2</sub>/1 mM-dithiothreitol/20 mM-Tris/HCl, pH 7.6, as described in the preceding paper (Cox & Hirst, 1976). The peak fractions were combined and the subparticles were collected by centrifuging for 17 h at 40000 rev/min (MSE 8 × 25 rotor). The pellets were resuspended in 0.1 M-NH<sub>4</sub>Cl/2 mM-MgCl<sub>2</sub>/15% (v/v) glycerol/1 mM-dithiothreitol/0.02 M-Tris/HCl, pH 7.6. This solvent, subsequently referred to as 'storage buffer', is derived from Falvey & Staehelin (1970). Typical concentrations were 15 mg/ml of L-subparticles and

8 mg/ml of S-subparticles. The final yield from approx. 200 mg of polyribosomes was 65 mg of L-subparticles and 34 mg of S-subparticles. Occasionally a third component travelling ahead of the L-subparticle peak was noted, and these fractions were pooled separately. It appears that this component is a dimer of the L-subparticle, since a single RNA component (*S*<sub>20,w</sub> approx. 28S) was found on analytical centrifugation after the dilute solution (40 μg/ml) was made 1% in sodium dodecyl sulphate. The subparticles were kept indefinitely at –80°C. The presence of glycerol in the buffer was beneficial; otherwise the subparticles lost activity within 2–3 weeks on storage. Two variations of these methods were used successfully in the early stages of this work. First the polyribosome fraction was incubated for 2 h at 37°C in the standard cell-free system for protein synthesis to produce run-off 80S ribosomes, and secondly the subparticles were recovered from the gradient fractions by first raising the concentration of MgCl<sub>2</sub> to 8 mM, then adding 0.6 vol. of methanol (or ethanol) at 0°C. The precipitate was coagulated at –12°C and recovered by centrifuging at low speed (approx. 1500g). Neither of these variations diminished the activity of the subparticles in either polyphenylalanine synthesis or in the puromycin reaction.

### Polyphenylalanine synthesis by the cell-free system

The incorporation of [<sup>14</sup>C]phenylalanine into acid-insoluble material by L-subparticles in the presence of poly(U), S-subparticles, supernatant factors etc. at 37°C was measured as described in the preceding paper (Cox & Hirst, 1976). Samples were generally 50 μl of 2.0 *E*<sub>260</sub> units of L-subparticles/ml in an assay mixture of 0.5 ml. Incubation was for 30–60 min, and occasionally there was a 10 min preincubation omitting the energy source.

### Assay of peptidyltransferase by the puromycin reaction

This assay measures the transfer of acetyl[<sup>3</sup>H]leucine groups from acetyl[<sup>3</sup>H]leucyl-tRNA to puromycin, as catalysed by isolated rabbit reticulocyte L-subparticle for 10 min at 20°C in a buffer containing 40 mM-magnesium acetate, 0.3 M-KCl, 40 mM-Tris/acetate, pH 7.5 (20°C), 30% (v/v) methanol and 1 mM-puromycin. These conditions are similar to those used to assay *Escherichia coli* ribosomes at 0°C in the fragment reaction (Greenwell *et al.*, 1974), and also similar to the assay of Thompson & Moldave (1974) for rat liver L-subparticles with acetylphenylalanyl-tRNA, except that their optimum concentration of MgCl<sub>2</sub> was 4 mM. Assay mixtures contained up to 0.5 *E*<sub>260</sub> unit (approx. 10 pmol) of L-subparticles in 50 μl, together with 0.2–0.3 pmol of acetyl[<sup>3</sup>H]leucyl-tRNA [specific radioactivity 17 Ci/mmol, prepared with bulk *E. coli* tRNA as described by Monro (1971)].

Incubation was carried out in small glass vials (13 mm × 46 mm, FBG-Trident Ltd., London SE7 8NP, U.K.) to allow solvent extraction and liquid scintillation counting of the product, acetyl<sup>[3H]</sup>-leucylpuromycin, directly within the same vial, as described by Greenwell *et al.* (1974). However, the enzyme incubation for 10 min at 20°C produced a high background value for extractable radioactivity (probably acetyl<sup>[3H]</sup>leucine methyl ester), which was therefore eliminated by using as a routine a modified alkaline-hydrolysis step, i.e. the reaction was terminated by addition of 100 μl of 0.25 M-NaOH in saturated NaNO<sub>3</sub> solution, followed by incubation for 10 min at 37°C. Finally 2.5 ml of solvent mixture (800 ml of toluene, 200 ml of ethyl acetate, 0.4 ml of acetic acid, 4.0 g of diphenyloxazole) was added with brief shaking as a combined neutralization, extraction and scintillation agent. The vials were placed inside standard vials and counted for radioactivity for at least 20 min in a Packard Tri-Carb model 2450 scintillation counter, at an efficiency of approx. 45%.

#### *Determination of protein by filter assay of Amido Black complex*

In addition to the method of Lowry *et al.* (1951), the concentration of ribosomal proteins in salt extracts of ribosomes, or in urea sample buffer for gel electrophoresis, was also measured by a modification of the method of Nakao *et al.* (1973). The sample (2–150 μl, containing 0–20 μg of protein) was adjusted to 0.2 M-MgCl<sub>2</sub> in 200 μl. An equal volume of 0.1% Amido Black dye in methanol/acetic acid/water (5:1:4, by vol.) was added and the mixture kept at room temperature (approx. 20°C) for 20 min to allow formation and precipitation of the protein-magnesium-dye complex. The mixture was diluted with 1.0 ml of methanol/acetic acid/water (5:1:4, by vol.) and filtered through a 13 mm GSWP Millipore filter. The filter was rinsed with 3 × 3.0 ml of 7% (v/v) acetic acid, and removed to a test tube, where the dye was eluted from the complex with 1.0 ml of solution containing 1% sodium dodecyl sulphate, 3% (w/v) NaHCO<sub>3</sub>, 50 mM-disodium EDTA in ethanol/water (1:1, v/v). The *E*<sub>630</sub> was then measured to estimate the protein content by comparison with standard solutions containing 0–20 μg of cytochrome *c*.

#### *Two-dimensional polyacrylamide-gel electrophoresis of ribosomal proteins*

Proteins were extracted from ribosomes by treatment with 0.1 M-magnesium acetate and 2 vol. of acetic acid for 40 min at 0°C. Two-dimensional polyacrylamide-gel electrophoresis was carried out essentially by the Howard & Traut (1973) modification of the original method of Kaltschmidt & Wittmann (1970).

The Martini & Gould (1971) system was also used as described by Godwin *et al.* (1974), when proteins were isolated and concentrated by precipitation with trichloroacetic acid.

#### *Materials*

L-<sup>[3H]</sup>Leucine (sp. radioactivity 17 Ci/mmol) and L-[U-<sup>14</sup>C]phenylalanine (sp. radioactivity 495 μCi/μmol) were from The Radiochemical Centre, Amersham, Bucks., U.K. Puromycin dihydrochloride, poly(U), phosphoenolpyruvate, GTP and horse heart cytochrome-*c* (type III) were supplied by Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey KT2 7BH, U.K. Pyruvate kinase and ATP were obtained from Boehringer Corporation (London) Ltd., London W5 2TZ, U.K. Other-wise chemicals were AnalaR quality from BDH Ltd., Poole, Dorset, U.K.

#### *Results*

##### *Poly(U)-directed polyphenylalanine synthesis*

The standard cell-free system described by Arnstein *et al.* (1964) was supplemented with a protein fraction containing elongation factors obtained from the reticulocyte cytosol by (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> fractionation. The amount of acid-insoluble polyphenylalanine synthesized was directly proportional to the concentration of L-subparticles over the range tested (0.02–0.1 *E*<sub>260</sub> unit, approx. 2–10 μg/assay). The composition of the assay mixture with respect to simple salts was 50 mM-KCl/5 mM-MgCl<sub>2</sub>/10 mM-GSH (reduced glutathione)/25 mM-Tris/HCl, pH 7.6, and the temperature of the assay was 37°C. The incorporation ranged from 15 to 50 mol of phenylalanine polymerized/mol of L-subparticles.

There was no loss in the activity of L-subparticles after 4 h at 0°C when either storage buffer or 0.5 M-NH<sub>4</sub>Cl / 10 mM–30 mM-MgCl<sub>2</sub> / 1 mM-dithiothreitol / 0.02 M-Tris/HCl, pH 7.6, was the solvent (Fig. 1*a*). Decreasing the MgCl<sub>2</sub> concentration to 2 mM (solvent 0.5 M-NH<sub>4</sub>Cl / 2 mM-MgCl<sub>2</sub> / 1 mM-dithiothreitol/0.02 M-Tris/HCl, pH 7.6) led, after 30 min at 0°C, to diminished activity in the standard cell-free system (Fig. 1*b*). The activity that was observed depended on the particular treatment of the subparticle fraction. Three procedures were followed, namely: (i) precipitation of the ribosome fraction with 0.7 vol. of ethanol, followed by resuspension in storage buffer (3 mg of L-subparticles/ml); (ii) the ribosome fraction was separated by centrifuging for 40 min at *g*<sub>max</sub>. 145 000 and approx. 0°C and resuspended in storage buffer (3 mg of L-subparticles/ml); (iii) the solution was diluted 15-fold with sample buffer (0.2 mg of L-subparticles/ml) to avoid separating any split proteins from core particles. The subparticle fractions obtained by procedures (i) and (ii)

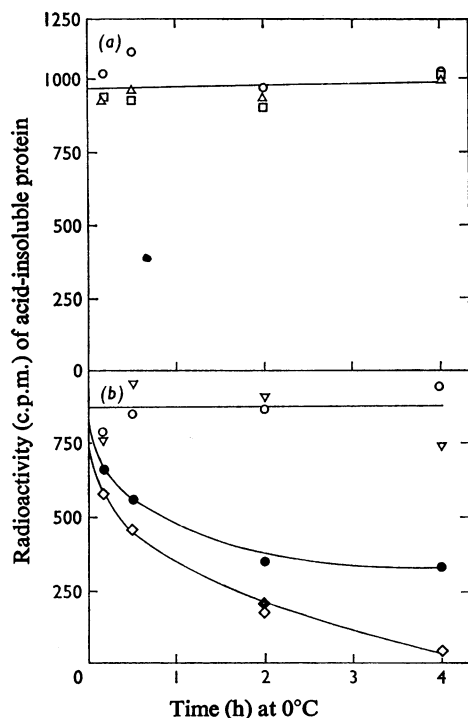


Fig. 1. Stability of polyphenylalanine-synthesis activity of rabbit L-subparticles at 0°C

L-subparticles (3 mg/ml) were treated as specified, and in each case samples were diluted to 0.2 mg/ml with sample buffer (0.25 M-sucrose/25 mM-KCl/1 mM-MgCl<sub>2</sub>/0.05 M-Tris/HCl, pH 7.6) and assayed in the standard cell-free system for 30 min at 37°C, after preincubation for 10 min at 37°C in the absence of the energy supply. (a) Some solvents in which activity was not affected: ○, storage buffer (0.1 M-NH<sub>4</sub>Cl/2 mM-MgCl<sub>2</sub>/1 mM-dithiothreitol/15% (v/v) glycerol/20 mM-Tris/HCl, pH 7.6); △, as for ○, except that MgCl<sub>2</sub> was increased to 12 mM; □, as for ○, except that NH<sub>4</sub>Cl was increased to 0.5 M and MgCl<sub>2</sub> was increased to 30 mM. (b) Experiments with controls demonstrating loss of activity owing to exposure to low Mg<sup>2+</sup> concentration: ▽, 0.5 M-NH<sub>4</sub>Cl/10 mM-MgCl<sub>2</sub>, otherwise as for storage buffer; ●, 0.5 M-NH<sub>4</sub>Cl/2 mM-MgCl<sub>2</sub>, otherwise as for storage buffer; ◇, as for ● but particles isolated by precipitation with 8 mM-MgCl<sub>2</sub> plus 0.7 vol. of ethanol for 1 h at -12°C, followed by centrifugation at 1500g and resuspension of the pellet in storage buffer; ◆, as for ●, but particles isolated by centrifugation at 105 000g<sub>av.</sub> for 2 h and resuspension of the pellet in storage buffer.

had similar activities in the poly(U)-directed synthesis of polyphenylalanine. The subparticle fraction was more active in polyphenylalanine synthesis when procedure (iii) was followed (see Fig. 1b).

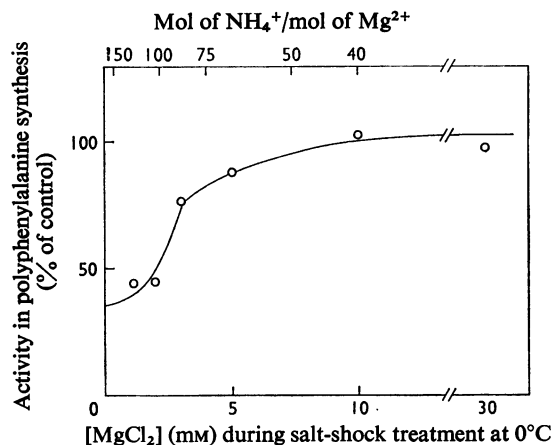


Fig. 2. Effect of MgCl<sub>2</sub> concentration during the salt-shock treatment on the inactivation of L-subparticles

L-subparticles were kept at 0°C for 1 h in 0.5 M-NH<sub>4</sub>Cl at the concentration of MgCl<sub>2</sub> indicated (abscissa). The samples were then diluted 15-fold with sample buffer and assayed for 30 min at 37°C in the standard cell-free system. Both the concentration of MgCl<sub>2</sub> in the 'salt-shock' buffer and the effective ratio (NH<sub>4</sub><sup>+</sup>+Tris)/MgCl<sub>2</sub> are indicated. The latter ratio includes an allowance for Mg<sup>2+</sup> contributed by L-subparticles.

#### Effect of MgCl<sub>2</sub> concentration during the salt-shock treatment on the activity of L-subparticles

Samples of L-subparticles were kept at 0°C for 1 h in 0.5 M-NH<sub>4</sub>Cl/2–30 mM-MgCl<sub>2</sub>/1 mM-dithiothreitol/0.02 M-Tris/HCl, pH 7.6. The samples were diluted 15-fold with sample buffer and then assayed in the standard cell-free system. There was little or no loss of activity when the concentration of added MgCl<sub>2</sub> was 10 mM or greater during the salt-shock treatment, but the activity was minimum in 2 mM-MgCl<sub>2</sub> (Fig. 2). The ribosomes themselves probably contribute to the absolute concentration of Mg<sup>2+</sup>. At a concentration of 30E<sub>260</sub> units of L-subparticles/ml the concentration of nucleotides is 4 mM, so that the concentration of Mg<sup>2+</sup> contributed by the ribosome fraction is likely to be in the range 2–4 mM (cf. Petermann & Pavlovic, 1967). Thus the true concentration of Mg<sup>2+</sup> could be 2–4 mM higher than the concentration of added MgCl<sub>2</sub>. When the nominal concentration of Mg<sup>2+</sup> falls so that there is nominally less than 1 Mg<sup>2+</sup> ion per 50 NH<sub>4</sub><sup>+</sup> ions, there appears to be an adverse effect on L-subparticles.

#### Kinetics of polyphenylalanine synthesis

The kinetics of polyphenylalanine synthesis were not the same for the control and 'shocked' L-subparticles. The main difference was the pronounced

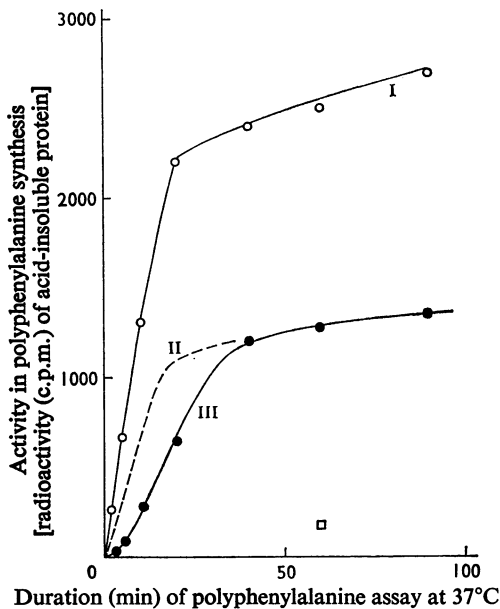


Fig. 3. Effect of  $\text{NH}_4\text{Cl}$  shock treatment on the time-course of the standard assay for protein synthesis

○, Control L-subparticles (3 mg/ml) in storage buffer diluted to 0.2 mg of L-subparticles/ml with sample buffer; ●, 'shocked' L-subparticles (3 mg/ml) in storage buffer with  $[\text{NH}_4\text{Cl}]$  increased to 0.5M, kept at 0°C for 2h then diluted to 0.2 mg of L-subparticles/ml with sample buffer. I, control L-subparticles; II, control L-subparticles normalized {curve I  $\times$  [c.p.m. after 90 min (curve III)/c.p.m. after 90 min (curve I)]} to the same final incorporation as 'shocked' L-subparticles; III, 'shocked' L-subparticles; □, control L-subparticles incubated without energy source.

delay in polyphenylalanine synthesis that was found after exposure to 2mM- $\text{MgCl}_2$  and which was not found for the control (see Fig. 3). It was inferred that L-subparticles inactivated by exposure to low concentrations of  $\text{MgCl}_2$  were re-activated during the incubation at 37°C. The ionic conditions of the assay appear favourable for re-activation, judged by the ratio  $\text{Mg}^{2+}$ /univalent cations, which is 1:15 in the assay but 1:260 in the low- $\text{MgCl}_2$  buffer.

Raising the concentration of  $\text{MgCl}_2$  from 2mM back to 10mM at 0°C was not sufficient to restore activity. However, activity was recovered (see Fig. 4) by first raising the concentration of  $\text{MgCl}_2$  and then incubating at 37°C (the buffer was 0.5M- $\text{NH}_4\text{Cl}$ /10–30mM -  $\text{MgCl}_2$ /1mM - dithiothreitol/0.02M - Tris/HCl, pH7.6).

Exposure to low concentrations of  $\text{MgCl}_2$  had a more noticeable effect on the puromycin reaction than on the ability to synthesize polyphenylalanine

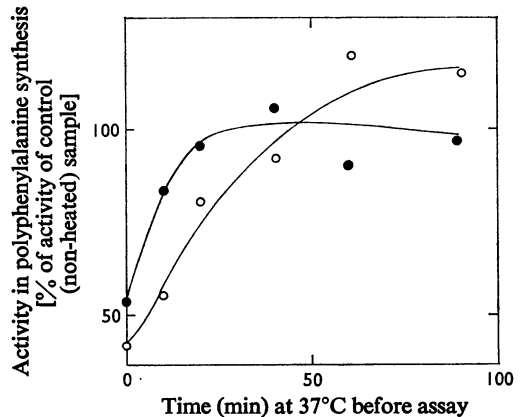


Fig. 4. Re-activation of 'shocked' L-subparticles by preincubation at 37°C in the presence of 10–30mM- $\text{MgCl}_2$ . L-subparticles (3 mg/ml) in storage buffer made 0.5M in  $\text{NH}_4\text{Cl}$  ('shocked') were kept at 0°C for 2h.  $\text{MgCl}_2$  was then added to a portion to a final concentration of either 10mM (○) or 30mM (●). A sample was kept at 37°C for up to 90min, cooled and then diluted to 0.2mg of L-subparticles/ml with sample buffer. The sample was then assayed for 90min at 37°C in the standard cell-free system after a preincubation for 10 min at 37°C in the absence of an energy source. The control (100%) was a sample of the stock non-treated solution.

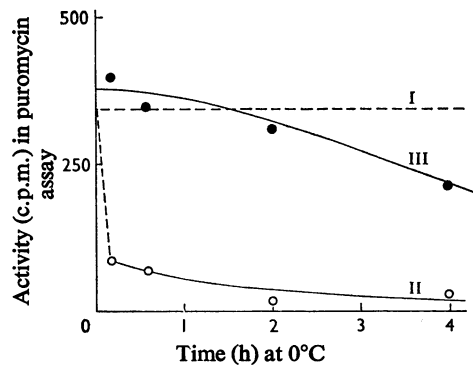


Fig. 5. Recovery of the ability of L-subparticles to function in the puromycin reaction after  $\text{NH}_4\text{Cl}$  shock treatment

(I) Activity of control L-subparticles (3 mg/ml) in 0.5M- $\text{NH}_4\text{Cl}$ /30mM- $\text{MgCl}_2$  kept at 0°C. (II) ○, Activity of L-subparticles (3 mg/ml) in 0.5M- $\text{NH}_4\text{Cl}$ /2mM- $\text{MgCl}_2$  kept at 0°C for the time indicated. (III) ●, Re-activated sample, i.e. a portion of the 'shocked' sample was made 30mM in  $\text{MgCl}_2$  (final concn. 0.5M- $\text{NH}_4\text{Cl}$ /30mM- $\text{MgCl}_2$ ) and kept at 37°C for 30 min, cooled, then assayed for peptidyl-transferase activity at 20°C.

(compare Fig. 1b and Fig. 5). The two assays were in closer agreement if the ratio of the initial rates of polyphenylalanine synthesis of control and 'shocked'

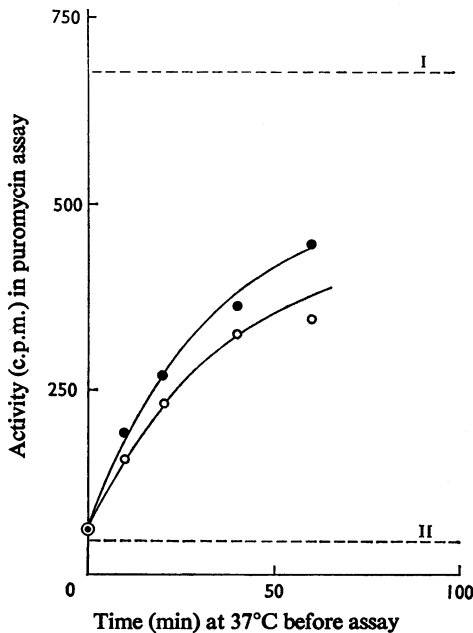


Fig. 6. Time-course of recovery of puromycin reaction activity of rabbit L-subparticles after  $\text{NH}_4\text{Cl}$  shock treatment

L-subparticles (3 mg/ml) were kept for 2 h at  $0^\circ\text{C}$  in storage buffer made 0.5M in  $\text{NH}_4\text{Cl}$ . Portions were adjusted to 10mM- $\text{MgCl}_2$  or 30mM- $\text{MgCl}_2$  and kept at  $37^\circ\text{C}$  for up to 60 min. The samples were cooled in ice and then assayed for peptidyltransferase activity: I, activity of control ribosomes (3 mg/ml) in 0.5M- $\text{NH}_4\text{Cl}$ /30mM- $\text{MgCl}_2$ ; II, activity of 'shocked' ribosomes kept at  $0^\circ\text{C}$  for 2 h in 0.5M- $\text{MgCl}_2$ ;  $\circ$ , 'shocked' solution adjusted to 10mM- $\text{MgCl}_2$  and kept at  $37^\circ\text{C}$  as indicated;  $\bullet$ , 'shocked' solution adjusted to 30mM- $\text{MgCl}_2$  and kept at  $37^\circ\text{C}$  for the times shown. Samples were also assayed for their ability to function in poly(U)-directed polyphenylalanine synthesis (see Fig. 4).

samples were compared with the ratio of the activities of control and shocked samples in the puromycin reaction. The difference between the two assays is also seen by comparing Fig. 4 and Fig. 6, where samples of a 'shocked' solution of L-subparticles were assayed separately for polyphenylalanine synthesis and the puromycin reaction during the course of re-activation at  $37^\circ\text{C}$ . The two assays agree provided that allowance is made for re-activation of the ribosome fraction in the standard cell-free system.

In other words the puromycin reaction gave a more correct measure of the inactivation of the peptidyltransferase centre caused by the salt treatment described in the present paper. The polyphenylalanine assay gave an underestimate because of re-activation of the L-subparticle during the incubation for 30 min

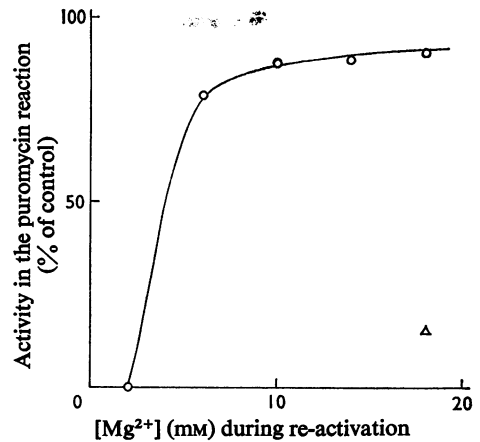


Fig. 7. Influence of  $\text{MgCl}_2$  concentration on the re-activation of L-subparticles by incubation at  $37^\circ\text{C}$ , as measured by the puromycin reaction

L-subparticles (3 mg/ml) were inactivated for 2 h at  $0^\circ\text{C}$  in storage buffer made 0.5M in  $\text{NH}_4\text{Cl}$ . Portions (100  $\mu\text{l}$ ) were supplemented with magnesium solutions (2  $\mu\text{l}$ ) to the concentrations shown, and then incubated at  $37^\circ\text{C}$  ( $\circ$ ) or  $0^\circ\text{C}$  ( $\Delta$ ) for 1 h, before assay of samples for puromycin-reaction activity. Control L-subparticles were maintained at  $0^\circ\text{C}$  in storage buffer until assayed.

at  $37^\circ\text{C}$ , whereas the conditions of the puromycin reaction (10 min at  $20^\circ\text{C}$ ) are less favourable for re-activation (see Fig. 8 below).

#### Dependence on $\text{MgCl}_2$ concentration of the re-activation at $37^\circ\text{C}$ of salt-shocked L-subparticles

Just as the stability of the L-subparticle at  $0^\circ\text{C}$  was found to depend on  $\text{MgCl}_2$  concentration, so also was the re-activation of peptidyltransferase activity at  $37^\circ\text{C}$ . There was no re-activation when the cation ratio was 0.5M- $\text{NH}_4\text{Cl}$ /2mM- $\text{MgCl}_2$ , but re-activation was obtained (up to 90% of the original activity) as the concentration of  $\text{MgCl}_2$  approached 10mM or greater (see Fig. 7).

#### Temperature-dependence of the re-activation of peptidyltransferase activity

L-subparticles were salt-shocked by exposure to 0.5M -  $\text{NH}_4\text{Cl}$ /2mM -  $\text{MgCl}_2$ /1mM - dithiothreitol/0.02M-Tris/HCl, pH 7.6. The  $\text{MgCl}_2$  concentration was restored to 10mM and the solutions were kept at  $0^\circ$ ,  $20^\circ$  or  $37^\circ\text{C}$  for periods up to 90 min. There was no re-activation of peptidyltransferase activity at  $0^\circ\text{C}$  and little (no more than 10%) after 90 min at  $20^\circ\text{C}$  (Fig. 8). More than 50% of activity was recovered

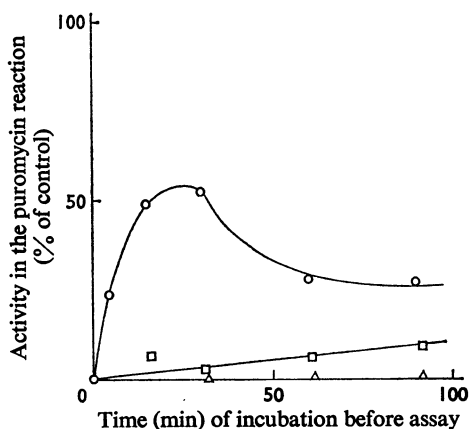


Fig. 8. Effect of temperature on the time-course of the re-activation of L-subparticles

L-subparticles (3 mg/ml) were inactivated for 2 h at 0°C in storage buffer made 0.5 M in  $\text{NH}_4\text{Cl}$ . The reaction mixture was supplemented with  $\text{Mg}^{2+}$  to 10 mM, and portions were incubated at 37°C (○), 20°C (□) or 0°C (△). Samples were removed at the times indicated, quickly frozen in puromycin-reaction assay mixture, and stored at -20°C until the end of the time-course. They were then thawed and assayed by the puromycin reaction, together with a control which had also been frozen and thawed.

after heating to 37°C. The shape of the curve at 37°C, i.e. the maximum recovery of activity after 30 min and the adverse effect of further heating at this temperature, is consistent with an adverse reaction, e.g. hydrolysis, competing with the re-activation process. However, this decline in activity is not always observed.

The limited extent of re-activation at 20°C and the greater re-activation noticed at 37°C are observations that agree with the notion that the salt-shocked L-subparticles are re-activated during the 30 min assay of poly(U)-directed polyphenylalanine synthesis at 37°C but not in the puromycin reaction (10 min at 20°C), where the highly aggregated state of the subunits in the presence of 30% (v/v) methanol may also affect their ability to be re-activated.

#### Effect of exposure to low concentrations of $\text{MgCl}_2$ on the protein moiety of L-subparticles

In principle the separation of a split-protein fraction free from core particles may be achieved by differential centrifugation. As a check it was noted that cytochrome *c* added to the solution remained largely in the supernatant fraction, which was free of ribosomes as judged by  $E_{260}$  measurements. The amount of protein found in the supernatant fraction

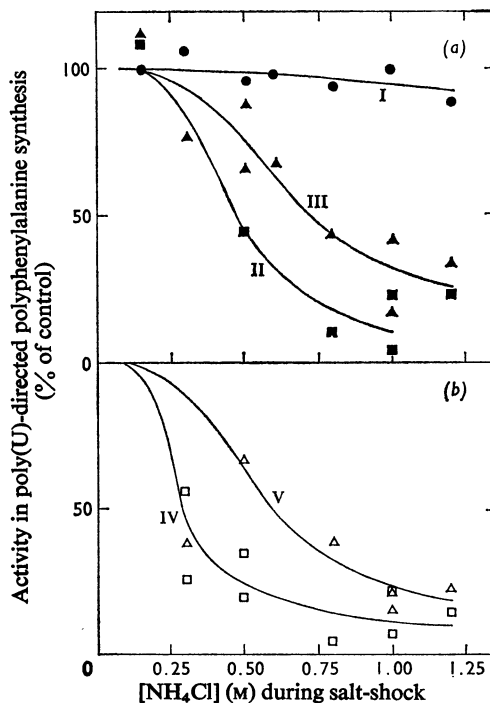


Fig. 9. Effect of re-activation procedure on the activity in polyphenylalanine synthesis of both L-subparticle salt-shock mixtures and isolated salt-treated particles prepared at 0.1-1.2 M- $\text{NH}_4\text{Cl}$

The Figure summarizes several experiments in which L-subparticles (3 mg/ml) were kept for 2 h at 0°C in storage buffer with the  $\text{NH}_4\text{Cl}$  concentration adjusted to a value in the range 0.1-1.2 M, but with  $\text{MgCl}_2$  kept at 2 mM. Control samples were protected against salt shock by the presence of 20 mM- or 30 mM- $\text{Mg}^{2+}$  (curve I). Samples of the reaction mixtures were assayed before (curve II) and after (curve III) re-activation. The L-subparticle fraction was isolated as described below and was also assayed before (curve IV) and after (curve V) re-activation. In the last step after treatment and before assay the samples were diluted to 0.2 mg of L-subparticles/ml with sample buffer. (a) ■, L-subparticle reaction mixtures assayed without a re-activation step; ▲, L-subparticle solutions assayed after re-activation by adjustment of  $\text{NH}_4\text{Cl}$  to 0.5 M (by dilution with 20 mM-Tris/HCl, pH 7.6, or addition of  $\text{NH}_4\text{Cl}$  solutions) and  $\text{MgCl}_2$  to 20 mM, followed by incubation at 37°C for 40 min; ●, control mixtures protected against salt-shock inactivation by the presence of 20 mM- or 30 mM- $\text{MgCl}_2$ . (b) □, L-subparticle fraction isolated from salt-shock mixture (by centrifugation at 100000  $g_{av}$ . and 4°C for 30 min, or by precipitation with 8 mM- $\text{MgCl}_2$  and 0.7 vol. of ethanol), resuspended in 0.5 M- $\text{NH}_4\text{Cl}$ /2 mM- $\text{MgCl}_2$ /1 mM-dithiothreitol/15% (v/v) glycerol/0.02 M-Tris/HCl, pH 7.6 at 0°C, and assayed; △, the salt-shocked, isolated and resuspended L-subparticles were subjected to a re-activation step at 37°C for 40 min after adjustment of  $\text{MgCl}_2$  to 20 mM.

was small, no more than  $3 \pm 2\%$ . Attempts by two-dimensional gel electrophoresis to identify proteins of the supernatant fractions gave negative results; we could find no positive evidence for the loss of protein. However, we cannot exclude the possibility that some of the protein moiety (no more than  $5\%$ ) is lost.

#### *Exposure of L-subparticles to higher concentrations of $\text{NH}_4\text{Cl}$*

To provide a frame of reference for the inactivation-re-activation studies, the conditions of the shock treatment were extended to cover a wider range of  $\text{NH}_4\text{Cl}$  concentrations while the concentration of  $\text{MgCl}_2$  was kept at 2 mM. The data are summarized in Fig. 9. The principal result is that the capacity of the 'shocked' fraction to become re-activated diminished as the  $\text{NH}_4\text{Cl}$  concentration increased. Ribosomes that were not exposed to low concentrations of  $\text{MgCl}_2$  were scarcely affected by the re-activation procedure (Fig. 9a). Limited re-activation was obtained at 0.8–1.2 M- $\text{NH}_4\text{Cl}$ . The activity of the ribosome fraction separated by precipitation with 0.7 vol. of ethanol or by differential centrifuging tended to be less than the activity observed when no attempt was made to separate core particles and split proteins. The pellets were capable of being re-activated. Protein assays on the supernatant fraction freed from subribosomal particles revealed positive evidence that protein was lost as the  $\text{NH}_4\text{Cl}$  concentration was increased, namely that no more than  $6 \pm 3\%$  protein was lost at 0.6 M- $\text{NH}_4\text{Cl}/2\text{ mM-MgCl}_2$ , approx.  $10 \pm 3\%$  protein was lost at 0.8 M- $\text{NH}_4\text{Cl}/2\text{ mM-MgCl}_2$ , approx.  $10 \pm 3\%$  was lost at 1 M- $\text{NH}_4\text{Cl}/2\text{ mM-MgCl}_2$  and approx.  $20 \pm 3\%$  was lost after exposure to 1.2 M- $\text{NH}_4\text{Cl}/2\text{ mM-MgCl}_2$ . The formation of protein-deficient core particles at higher  $\text{NH}_4\text{Cl}$  concentrations, and their reassembly, is described in the following paper (Cox & Greenwell, 1976).

#### *Difference spectrometry*

The u.v.-absorbance spectrum of L-subparticles in the range 240–330 nm is affected by changes in the secondary structure of the RNA component, which is the principal absorbing species at these wavelengths. Small differences in the spectrum may be measured accurately with modern spectrophotometers, and there is a basis for interpreting changes in the u.v.-absorbance spectrum (Cox, 1966, 1971). For practical reasons the change in spectrum was measured when the concentration of  $\text{Mg}^{2+}$  was restored at  $37^\circ\text{C}$  from 2 mM to 20 mM- $\text{MgCl}_2$  (see Fig. 10a). The addition of  $\text{Mg}^{2+}$  led to a decrease in  $E_{260}$  of approx. 2% (i.e. a measured change in extinction of about 0.02). The difference spectrum was characteristic of an increase in A·U base-pairs, with a minimum at 260 nm, a very

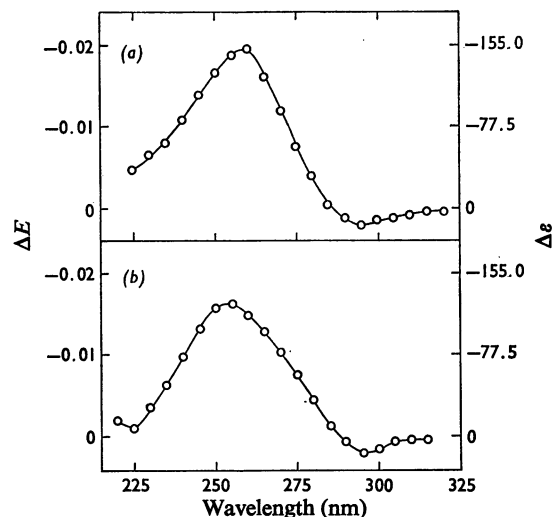


Fig. 10. Difference in the spectrum of L-subparticles and L-rRNA found on increasing  $\text{MgCl}_2$  from 1–2 mM to 15–20 mM

(a) L-subparticles (3 mg/ml) in storage buffer made 0.5 M in  $\text{NH}_4\text{Cl}$  were inactivated at  $0^\circ\text{C}$  for 2 h. The salt-shocked subparticles were then diluted to  $E_{260} = 1.0$  in 0.5 M- $\text{NH}_4\text{Cl}/2\text{ mM-MgCl}_2/20\text{ mM-Tris/HCl}$ , pH 7.6, 3.0 ml portions were placed in 1 cm cuvettes in the Cary 118 Spectrophotometer and a baseline scanned at  $37^\circ\text{C}$  (full-scale deflexion = 0.05). Then 3.0 M- $\text{MgCl}_2$  (20  $\mu\text{l}$ ) and water (20  $\mu\text{l}$ ) were added to the sample and reference cuvettes respectively, and the difference spectrum was measured within 5 min, and monitored for a further 90 min. (b) Rabbit L-rRNA was isolated by precipitation as the guanidinium salt, and dialysed overnight against 0.35 M-KCl/10 mM-Tris/HCl, pH 7.2. Samples (3 ml,  $E_{260} = 1.0$ ) were placed in cuvettes and the baseline was scanned at  $20^\circ\text{C}$ . Then 3 M- $\text{MgCl}_2$  was added to one sample and an equivalent volume of water (or more dilute  $\text{MgCl}_2$ ) was added to the other and the difference spectrum was measured. The difference in the spectrum when the concentration of  $\text{MgCl}_2$  was 1 mM in the reference cell and 15 mM in the sample cell is given in the Figure [see also the preceding paper, Cox & Hirst (1976)].

small change at 280 nm and a small increase at 290 nm. The change in the spectrum was observed to occur within the time taken (5 min) for the difference spectrum to be recorded. No further change in  $E_{260}$  was found during the next 90 min. The change in the spectrum corresponds to the net formation of approx. 100 A·U base-pairs per molecule of the major rRNA species of the larger subparticle (L-subparticles). This estimate is based on  $\epsilon_p$  of  $7750\text{ litre}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$  at 260 nm for L-rRNA (Cox, 1970), a decrease in  $\epsilon_p$  at 260 nm of  $4500\text{ litre}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$  on the formation of an A·U base-pair (Cox, 1970), and a mol.wt. of 1720000 (Loening, 1968). A similar change was



noted when  $Mg^{2+}$  was added to isolated L-rRNA (Fig. 10b). ( $\epsilon_p$  is the molar extinction coefficient per g-atom of phosphorus in an RNA species.)

#### Sedimentation-velocity studies

The value of  $s_{20,w}^0$  was  $60 \pm 2.5S$  for L-subparticles (cf. Dintzis *et al.*, 1958; Ts'o & Vinograd, 1961) in several buffers in which the activity of L-subparticle in polyphenylalanine synthesis is preserved, e.g. 1 mM- $MgCl_2$ /0.01 M-sodium phosphate buffer, pH 7.2, 1 mM- $MgCl_2$ /0.025 M-KCl/0.05 M-Tris/HCl, pH 7.6, and 5 mM- $MgCl_2$ /0.025 M-KCl/0.05 M-Tris/HCl, pH 7.6. Measurements were made at seven different ribosome concentrations in the range 40  $\mu$ g–10 mg of L-subparticles/ml and  $s_{20,w}$  was obtained by extrapolation to zero concentration. The values of  $s_{20,w}^0$  and  $s_{20,w}$  at a concentration of 40  $\mu$ g/ml were the same within experimental error. When 0.5 M- $NH_4Cl$ /22 mM- $MgCl_2$ /0.02 M-Tris/HCl, pH 7.6, was the solvent a value for  $s_{20,w}$  of  $58 \pm 1S$  was found. A smaller value,  $s_{20,w} = 52 \pm 1S$ , was obtained for L-subparticles in 0.5 M- $NH_4Cl$ /2 mM-Tris/HCl, pH 7.6, at 0–18°C. The value  $s_{20,w} = 52 \pm 1S$  was also observed when the  $Mg^{2+}$  concentration was brought back to 22 mM without incubation at 37°C. After 1 h at 37°C,  $s_{20,w}$  increased from  $52 \pm 1S$  to  $56 \pm 1S$  compared with  $58 \pm 1S$  for the control non-treated sample kept in 0.5 M- $NH_4Cl$ /22 mM- $MgCl_2$ /0.02 M-Tris/HCl, pH 7.6. Thus exposure to low  $Mg^{2+}$  concentrations appears to increase the hydrodynamic volume of the subparticle. The original  $s_{20,w}$  value was approached after the

$Mg^{2+}$  concentration was restored and the solution kept at 37°C for 1 h. The results are summarized in Table 1.

The sedimentation profile for L-rRNA was also measured (see Table 1) and a single component with  $s_{20,w} = 38S$  was observed in 0.5 M- $NH_4Cl$ /0.02 M-Tris/HCl, pH 7.6, compared with  $s_{20,w} = 40 \pm 1S$  in 0.5 M- $NH_4Cl$ /2 mM- $MgCl_2$ /0.02 M-Tris/HCl, pH 7.6. Raising the concentration of  $Mg^{2+}$  to 22 mM did not alter the  $s_{20,w}$  but did lead to aggregation. Thus  $Mg^{2+}$  concentrations had more effect on  $s_{20,w}$  of the subparticle than on the rRNA moiety, at this concentration (0.5 M) of  $NH_4Cl$ .

#### Discussion

The results show that rabbit L-subparticles are stable at 0°C for at least 4 h in 0.5 M- $NH_4Cl$ /10–30 mM- $MgCl_2$ . The stability is dependent on the concentration of  $Mg^{2+}$ , and activity is lost as the ratio  $NH_4^+/Mg^{2+}$  in the solvent is altered so that there is less than 1  $Mg^{2+}$  ion per 40  $NH_4^+$  ions. The true concentration of  $Mg^{2+}$  is higher than this by the amount bound originally to L-subparticles in storage buffer. Petermann & Pavlovec (1967) showed that the amount of  $Mg^{2+}$  bound by rat liver ribosomes approaches 0.5  $Mg^{2+}$  ion/atom of RNA phosphorus in buffers in which ribosomes are usually stored. In our experiments the ribosome fraction could contribute sufficient  $Mg^{2+}$  to raise the concentration by 2 mM. Weiss *et al.* (1973) examined the

Table 1. Sedimentation properties of L-subparticles and L-rRNA in solutions of different  $Mg^{2+}$  concentrations

L-subparticles (0.05 mg/ml) were kept for 2 h at 0°C in 0.5 M- $NH_4Cl$ /0.02 M-Tris/HCl, pH 7.6, containing  $MgCl_2$  as indicated, and subsequently treated as shown, before the sedimentation coefficient of the subparticle was measured as described in the Methods section. For comparison, values for the puromycin reaction are included to show the range of peptidyltransferase activity observed in separate but similar experiments. These experiments differed in that the L-subparticle concentration was 3 mg/ml and the buffers contained 1 mM-dithiothreitol.

| Species        | Treatment  | $Mg^{2+}$ in solvent (mM) | $s_{20,w}$ (S)          | Activity in puromycin reaction after treatment (% of control) |
|----------------|--|---------------------------|-------------------------|---|
| L-subparticles | Control (no treatment)   | 22                        | $58 \pm 1S$ +dimer      | 100   |
|                | Exposed to 2 mM- $MgCl_2$ for 2 h at 0°C   | 2                         | $52 \pm 1S$ +dimer      | 2–10  |
|                | Exposed to 2 mM- $MgCl_2$ then adjusted to 22 mM- $MgCl_2$ at 18°C                       | 22                        | $52 \pm 1S$ +aggregates | 5–15  |
|                | Exposed to 2 mM- $MgCl_2$ then adjusted to 22 mM- $MgCl_2$ and heated for 10 min at 37°C | 22                        | 52–56S (broad boundary) | 35–40   |
|                | Exposed to 2 mM- $MgCl_2$ then adjusted to 22 mM- $MgCl_2$ and heated for 1 h at 37°C    | 22                        | $56 \pm 1S$ +dimer      | 30–90   |
| L-rRNA         | L-subparticles treated with 1% sodium dodecyl sulphate at 18°C                           | 0                         | $38 \pm 1S$             | —   |
|                | L-subparticles treated as above  | 2                         | $40 \pm 1S$             | —   |
|                | L-subparticles treated as above  | 22                        | $40 \pm 1S$ +aggregates | —   |

quantity of  $Mg^{2+}$  bound by *E. coli* L-subparticles when  $NH_4^+$  and  $K^+$  concentrations were varied, and they noted that the activity in poly(U)-directed polyphenylalanine synthesis diminished at a ratio lower than 0.2  $Mg^{2+}$  ion/atom of RNA phosphorus for  $Mg^{2+}$  bound to L-subparticle. No re-activation of *E. coli* L-subparticle was achieved by Weiss *et al.* (1973). We have not measured the amount of ribosome-bound  $Mg^{2+}$  but we have sought the concentration of  $Mg^{2+}$  in the solvent needed to maintain full activity.

The loss of activity in the puromycin assay brought about by decreasing the  $Mg^{2+}$  concentration shows that peptidyltransferase activity was lost. The loss of peptidyltransferase activity is sufficient to account for the loss of activity in the polyphenylalanine assay. Other activities, such as the ability to bind to the small subribosomal particle, or the capacity to bind aminoacyl-tRNA, peptidyl-tRNA and elongation factors could also have been affected, but we did not study these partial reactions.

Exposure to low concentrations of  $Mg^{2+}$  appears to inactivate the peptidyltransferase site without releasing protein. However, the sensitivity of the protein assay is too low to exclude this possibility completely. Miskin *et al.* (1970) showed the *E. coli* L-subparticles may be inactivated by changing the ionic environment. The effects were seen by changing the univalent cation so that L-subparticles were deprived of  $NH_4^+$  and not  $Mg^{2+}$ . Re-activation was achieved by restoring the original concentration of  $NH_4^+$  and heating, e.g. at 40°C. Conversely, our work shows that in the presence of  $NH_4^+$  the activity of rabbit L-subparticles depends critically on the concentration of  $Mg^{2+}$ . A ratio of at least one  $Mg^{2+}$  ion/40  $NH_4^+$  ions appears to be necessary to maintain the activity of the subparticle. Zamir *et al.* (1971) have shown that *E. coli* S-subparticle also may be inactivated reversibly, by depletion of either  $Mg^{2+}$  or  $NH_4^+$ . In all these cases, restoration of function to the ribosomal subparticles is strongly dependent on temperature (cf. Fig. 8) and the presence of sufficient  $Mg^{2+}$ .

One explanation of the dependence of the integrity of L-subparticles on  $Mg^{2+}$  is that  $Mg^{2+}$  deprivation at 0°C leads to a metastable conformational change that is brought about because the conformation of the RNA moiety is governed by the concentration of  $Mg^{2+}$ . Evidence in support of this view has been obtained [see the preceding paper, Cox & Hirst (1976)], since it was found that the addition of  $MgCl_2$  to solutions of the isolated rRNA moiety of both rabbit and *E. coli* L-subparticles in 0.5M- $NH_4Cl$  (or 0.35M-KCl) led to (i) a decrease of approx. 2% in  $E_{260}$ ; (ii) a shift in the 'melting' profile ( $E_{260}$  against temperature) of about 5°C towards higher temperatures and (iii) an increase in  $s_{20,w}$  (see Table 1). These effects tended to a limit as the con-

centration of  $MgCl_2$  approached 10 mM. The difference spectrum of L-subparticles (Fig. 10) noted when the concentration of  $Mg^{2+}$  was increased from 2 mM to 10 mM at 37°C is in accord with these findings. The difference suggests that there is a net increase in the number of A·U base-pairs on raising the concentration of  $Mg^{2+}$  of approx. 100 A·U base-pairs/molecule (5100 nucleotides). The major rRNA moiety (L-rRNA) of rabbit L-subparticles has an uneven distribution of guanine nucleotides (e.g. Cox, 1966, 1970) owing to long tracts rich in guanine and cytosine residues (approx. 78% G+C; Cox *et al.*, 1973a), so that formation of A·U base-pairs on the addition of  $MgCl_2$  is probably confined to the non-(G+C)-rich regions of L-rRNA that are believed to have been conserved during the evolution of a wide range of species (Cox *et al.*, 1973a, 1976b).

Lowering the concentration of  $Mg^{2+}$  to 2 mM led to a decrease in  $s_{20,w}$  and indicated a partial unfolding of the subparticle. The effect is reversed by raising the concentration of  $Mg^{2+}$  and heating at 37°C. The change in  $s_{20,w}$  roughly parallels the loss and recovery of peptidyltransferase activity (Table 1). By contrast, the increase in A·U base-pairing was found to take place more rapidly, within 5 min of the addition of  $Mg^{2+}$ . We infer that the first effect of restoring the concentration of  $Mg^{2+}$  from 2 mM to 10–30 mM in the presence of 0.5M- $NH_4^+$  is to restore the native L-rRNA conformation in a way similar to that which occurs with isolated L-rRNA (although the secondary structure of the L-rRNA moiety in L-subparticles is modified by the protein moiety, as judged by 'melting' profiles; Cox *et al.*, 1973b). The net change in L-rRNA conformation on the addition of  $Mg^{2+}$  is small (an additional 6% of residues form A·U base-pairs), but it is emphasized that, even in the absence of  $Mg^{2+}$ , rabbit L-rRNA has a high degree of secondary structure, in which 80–90% of nucleotide residues are ordered (Cox, 1970); approx. 65% of residues may form base-pairs, and a further approx. 25% of residues contribute to ordered non-base-paired secondary structure, e.g. 'stacking' in single-stranded regions (Cox *et al.*, 1976a).

Once the native L-rRNA conformation is restored, the slower heat-dependent changes in subparticle conformation (indicated by the changes in  $s_{20,w}$ ) may then take place. This second process probably involves the relaxation of metastable interactions between the ribosomal components, as the proteins revert to their original interactions with the L-rRNA. This rearrangement results in restoration of function and a more compact particle structure, but no detectable further change in the RNA conformation as judged by spectrophotometry.

There is increasing evidence that rRNA is directly involved in various ribosomal functions, for example in tRNA binding (Erdmann *et al.*, 1973) and in mRNA recognition (Steitz & Jakes, 1975). For

*E. coli* peptidyltransferase, affinity labelling (Greenwell *et al.*, 1974; Breitmeyer & Noller, 1976) has demonstrated that parts of L-rRNA are sufficiently close to the active site of the enzyme to combine with reactive substrate analogues. Possibly therefore the rRNA may also have a direct role in the peptidyltransferase function. Our current observations indirectly support this view by drawing attention to the importance of L-rRNA conformation to the peptidyltransferase activity of rabbit ribosomes.

We thank Mrs. B. Higginson and Miss Hazel Barrett for valuable technical assistance.

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