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# A Study of the Influence of Magnesium Ions on the Conformation of Ribosomal Ribonucleic Acid and on the Stability of the Larger Subribosomal Particle of Rabbit Reticulocytes

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1. $Mg^{2+}$ was shown to affect the conformation of rRNA over the range 0.03–1.2M-KCl. The species studied were *Escherichia coli* S-rRNA and L-rRNA (the RNA moieties of the smaller and larger subribosomal particles respectively) and rabbit S-rRNA and L-rRNA. 2. The addition of $Mg^{2+}$ to rRNA in reconstitution buffer (0.35M-KCl/0.01M-Tris/HCl, pH7.2) at 20°C led to an increase in bihelical secondary structure through the formation of additional (mainly A·U) base-pairs (e.g. an additional approx. 58 A·U base-pairs per molecule of *E. coli* S-rRNA as judged by u.v. difference spectrophotometry and circular-dichroism measurements), to an increase in the stability of bihelical secondary structure by about 5°C, and to an increase in $s_{20,w}$. 3. Additional $Mg^{2+}$ was needed to effect the above, mentioned changes as the concentration of KCl was increased. The relation between rRNA conformation, KCl concentration and $MgCl_2$ concentration is given by the equation:

$$K_{10} = [N_{II}][K^+]^{1.3}/[N_I][Mg^{2+}] = 80 \pm 5$$

where $K_{10}$ is the equilibrium constant and $[N_I]$ and $[N_{II}]$ are the concentrations of nucleotides in the $Mg^{2+}$-free ($N_I$) and $Mg^{2+}$-stabilized ($N_{II}$) conformations. 4. The same equation may be used to describe the stability of the smaller and larger subribosomal particles of the rabbit, as measured by their ability to function in poly(U)-directed polyphenylalanine synthesis. Full activity was retained after exposure to 1.8M-$NH_4Cl$/45mM-$MgCl_2$ for 2h at 0°C. 5. It is suggested that rRNA has a 'native' conformation that is essential for the activity of the subparticle and that $Mg^{2+}$ contributes to ribosome stability by maintaining rRNA in this 'native' state. The application of this notion to the assembly and disassembly of functional subribosomal particles is discussed.

The native structure of ribosomes depends on a minimum concentration of $Mg^{2+}$ or an equivalent ion (see, e.g., Petermann & Pavlovec, 1967; Weiss & Morris, 1973; Cox *et al.*, 1976*a*) and also univalent cations (Hultin *et al.*, 1973). The possibility arises that $Mg^{2+}$ affects the conformation of the ribosome largely through its effect on the conformation of the rRNA component, since $Mg^{2+}$ binds to the rRNA moiety of ribosomes (Edelman *et al.*, 1960). The aim of the present work was to examine the effect of $Mg^{2+}$ on rRNA conformation as judged by spectroscopic methods and sedimentation-velocity studies and to relate it to the $Mg^{2+}$ requirement of ribosomes. The rRNA species studied were *Escherichia coli* S-rRNA, L-rRNA and rabbit S-rRNA and L-rRNA (S- and L-rRNA being the major RNA species of the smaller and larger subribosomal particles respectively).

A theory was devised that allows the dependence of ribosome stability to be expressed quantitatively in terms of the $Mg^{2+}$ concentration. The results provide evidence that the $Mg^{2+}$ requirement of ribosomes is closely related to the effect of $Mg^{2+}$ on rRNA conformation. This conclusion is based on two experimental approaches.

First, rRNA in components of reconstitution buffer (0.35M-KCl/10mM-Tris/HCl, pH7.2; Mizushima & Nomura, 1970) was titrated with $MgCl_2$, and conformational changes were followed by measuring the u.v.-absorption spectrum, 'melting' profiles, the c.d. (circular dichroism) spectrum and $s_{20,w}$.

It was noted that the addition of $MgCl_2$ to *E. coli* S-rRNA led to an increase in bihelical secondary structure of approx. 58 A·U base-pairs at 20°C, to an increase of approx. 5°C in the thermal stability of the bihelical parts, and to a decrease in hydrodynamic volume. The $Mg^{2+}$ requirement for rRNA conformation parallels the $Mg^{2+}$ requirement for the reconstitution of the subparticle (cf. Traub & Nomura, 1969). Comparable effects were found for the interaction of $MgCl_2$ with the other rRNA species studied. The titration of rRNA in low and high salt concentrations (0.03–1.2M-KCl) was followed spectrophotometrically; in all cases there was an increase in secondary structure on addition of $MgCl_2$.

Secondly, the effect of $Mg^{2+}$ on the stability of the larger subribosomal particle ('L-subparticle') of the rabbit in 0.5–2.25M-$NH_4Cl$ was measured by its activity in cell-free polyphenylalanine synthesis. An $Mg^{2+}/NH_4^+$ molar ratio of approx. 1:40 was needed

to preserve function. This requirement is similar to that found for rRNA, since an $Mg^{2+}/K^+$ molar ratio of 1:40 optimized additional A·U base-pair formation.

Finally, the stability of rabbit subribosomal particles towards shock treatment with $NH_4Cl$ for 2h at 0°C was examined when the amount of $Mg^{2+}$ was kept at an $Mg^{2+}/NH_4^+$ molar ratio of 1:40 to maintain the 'native' conformation of the rRNA moiety. Activity was diminished after treatment with 2M-$NH_4Cl$. The smaller subribosomal particle ('S-subparticle') was half inactivated after shock treatment with 3M-$NH_4Cl$; the L-subparticle was slightly less stable, the mid-point for inactivation occurring at 2.25M-$NH_4Cl$. Treatment with high concentrations of $NH_4Cl$ (>2M) in the presence of one $Mg^{2+}$ ion/40 $NH_4^+$ ions appears useful for producing core particles suitable for ribosome-reassembly experiments.

## Theoretical

We suppose that certain nucleotide sequences, a fraction, $s$, of the total, are sensitive to $Mg^{2+}$ so that the conformation of an individual nucleotide, N, is changed according to eqn. (1) from state I (represented as $N_I$) to state II (shown as $N_{II}$):

$$N_I + nMg^{2+} \rightleftharpoons N_{II} \quad (1)$$

If $x$ is the fraction of $Mg^{2+}$-sensitive residues in state II then the equilibrium constant (see eqn. 2) is given by eqn. (3):

$$K_1 = [N_{II}]/([N_I][Mg^{2+}]^n) \quad (2)$$

$$K_1 = x/[(1-x)[Mg^{2+}]^n] \quad (3)$$

The extinction per mol of nucleotide ($\varepsilon_\lambda$) is $\varepsilon_\lambda^I$ and $\varepsilon_\lambda^{II}$ for the two conformations and the reaction may be followed spectrophotometrically provided that $\varepsilon_\lambda^I \neq \varepsilon_\lambda^{II}$. The value of $\varepsilon_\lambda$ that is observed is given by eqn. (4):

$$\varepsilon_\lambda = (1-x)s\,\varepsilon_\lambda^I + xs\,\varepsilon_\lambda^{II} + (1-s)\varepsilon_\lambda^I \quad (4)$$

where $(1-s)\varepsilon_\lambda^I$ is the contribution to $\varepsilon_\lambda$ of the nucleotides that are not influenced by the concentration of $Mg^{2+}$.

The limiting values of $\varepsilon_\lambda$ are those found either in the absence of $Mg^{2+}$ ($\varepsilon_\lambda^0$) or in the presence of saturating amounts of the cation ($\varepsilon_\lambda^\infty$). In the former cases $x$ is zero, and in the latter case $x$ is unity, so that:

$$\varepsilon_\lambda^0 = s\varepsilon_\lambda^I + (1-s)\varepsilon_\lambda^I \quad (5)$$

$$\varepsilon_\lambda^\infty = s\varepsilon_\lambda^{II} + (1-s)\varepsilon_\lambda^I \quad (6)$$

The contribution of the residues that are not effected by $Mg^{2+}$ concentration is a constant, $(1-s)\varepsilon_\lambda^I$, so eqns. (4), (5) and (6) may be combined to give:

$$x = \frac{\varepsilon_\lambda - \varepsilon_\lambda^0}{\varepsilon_\lambda^\infty - \varepsilon_\lambda^0} \quad (7)$$

The term $\varepsilon_\lambda - \varepsilon_\lambda^0$ ($\Delta\varepsilon_\lambda$) is the change in extinction of the sample at wavelength $\lambda$ on addition of $Mg^{2+}$ and $\varepsilon_\lambda^\infty - \varepsilon_\lambda^0$ (termed $\Delta\varepsilon^\infty$) is the limiting change seen when the absorbance of the sample in the presence of an infinite amount of $Mg^{2+}$ is compared with the same sample without $Mg^{2+}$.

Solving eqn. (3) for $x$ and substitution into eqn. (7) leads to:

$$\frac{\Delta\varepsilon_\lambda^\infty}{\Delta\varepsilon_\lambda} - 1 = \frac{1}{K_1[Mg^{2+}]^n} \quad (8)$$

Assuming that an estimate of $\Delta\varepsilon_\lambda$ is available, a double-logarithmic plot (eqn. 9) gives both $K_1$, the equilibrium constant of the reaction, and $n$, the number of $Mg^{2+}$ ions bound per nucleotide in changing the conformation from state I to state II:

$$\log[(\Delta\varepsilon_\lambda^\infty/\Delta\varepsilon_\lambda - 1) - 1] = \log(1/K_1) - n\log[Mg^{2+}] \quad (9)$$

Eqn. (9) was solved by using a non-linear least-squares analysis and the initial assumption that $\Delta\varepsilon_\lambda^\infty$ was 10% higher than the maximum measured value of $\Delta\varepsilon_\lambda$. The values of $K_1$ and $n$ were estimated to be accurate to better than 20%.

Eqn. (1) describes the change in structure of RNA when the $Mg^{2+}$ concentration is the only parameter to change. The constants $K_1$ and $n$, of eqn. (2), would be expected to vary when the addition of $Mg^{2+}$ was repeated under different solvent conditions. In particular, the equilibrium described by eqn. (1) may be altered to include the effect of $K^+$ (see eqn. 10):

$$N_I + nMg^{2+} \rightleftharpoons Mg_nN_{II} + mK^+ \quad (10)$$

The equilibrium constant for this reaction, $K_{10}$, is given by eqn. (11):

$$K_{10} = \frac{[N_{II}][K^+]^m}{[N_I][Mg^{2+}]^n} \quad (11)$$

or

$$K_{10} = K_1[K^+]^m \quad (12)$$

where $K_1$ is the equilibrium constant for the interaction of $Mg^{2+}$ with RNA in the presence of a known amount of $K^+$. A double-logarithmic plot (see eqn. 13) of the equilibrium constant $K_1$ against $K^+$ concentration is linear with a gradient of $m$ and an intercept of $K_{10}$:

$$\log K_1 = \log K_{10} - m\log[K^+] \quad (13)$$

Another method of estimating $K_{10}$ is to measure the $Mg^{2+}$ concentration ($[Mg^{2+}]_{0.5}$) when $N_I$ and $N_{II}$

are present in equal amounts, so that eqn. (11) simplifies to eqn. (14):

$$K_{10} = \frac{[K^+]^m}{[Mg^{2+}]^n_{0.5}} \quad (14)$$

$Mg^{2+}$ screens the negatively charged diesterified phosphate residues more efficiently than $K^+$ (see Cox & Littauer, 1962). Thus $Mg^{2+}$ may function as $K^+$, but more efficiently, and it may also allow interactions between nucleotides that are not permitted by $K^+$, even in very high concentrations. Thus at a concentration of $x$M-KCl the fraction, $s$, of residues whose conformation is sensitive to $Mg^{2+}$ is given by eqn. (15):

$$s = ([N_I]+[N_{II}])/[N] = [f_{bh}(K^+, \text{max.}) - f_{bh}(K^+, x)] + f_N(Mg^{2+}) \quad (15)$$

where [N] is the concentration of nucleotides, $f_{bh}(K^+, \text{max.})$ is the maximum fraction of residues forming base-pairs at very high KCl concentration, $f_{bh}(K^+, x)$ is the fraction of residues forming base-pairs in $x$M-KCl, and $f_N(Mg^{2+})$ is the fraction of residues whose conformation is sensitive to $Mg^{2+}$ and not $K^+$. Thus $[N_I]$ and $[N_{II}]$ in eqn. (11) are related to the concentration of KCl through eqn. (15). $\varepsilon^0_\lambda$ will also depend on KCl concentration because $f_{bh}$ depends on KCl concentration; the magnitude of this effect is given by the term $[f_{bh}(K^+, \text{max.}) - f_{bh}(K^+, x)] \cdot \Delta\varepsilon_{bh}$, where $\Delta\varepsilon_{bh}$ is the average value for the change in $\varepsilon$ when a non-paired residue becomes base-paired. If $\varepsilon^\infty_\lambda$ is independent of KCl concentration and if $\varepsilon^0_\lambda$ decreases as the concentration of KCl increases, then the difference $(\varepsilon^\infty_\lambda - \varepsilon^0_\lambda)$ will also decrease on increasing the KCl concentration.

## Methods

### *General methods*

*U.v. spectrophotometry.* A Unicam SP.500 spectrophotometer was used for routine measurements. The 'melting' profiles were measured in a Unicam SP.700 spectrophotometer in which the sample cell-holder was a copper block that could be heated electrically to 95°C and maintained at a temperature within this region to better than 0.1°C. Values in extinction were corrected for changes in solute concentration owing to the thermal expansion of water. The reference cell was kept at 25°C.

U.v. difference spectra were measured in a Cary 118 spectrophotometer. The full scale was set to 0.05 so that differences in extinction could be measured to ±0.0005. The cell holders were connected in series to a Haake recirculating bath that allowed measurements to be made up to 60°C.

*Circular dichroism.* The c.d. of rRNA was measured in a 1cm-path-length cell ($E_{260} \simeq 1.0$) at room temperature (20°C) with a Rousset Jouan Dichrograph, model CD185. The results are expressed as c.d. $= (\varepsilon_L - \varepsilon_R)$, where $\varepsilon_L$ and $\varepsilon_R$ are respectively the values of $\varepsilon$ per g-atom of P for left and right circularly polarized light.

*Ultracentrifugation.* A Beckman model E ultracentrifuge fitted with u.v. optics was used for analytical studies. The camera was replaced by a photoelectric cell that scanned the slit, and the movement of the sedimenting species was followed by means of an *xy* recorder. Cells of 12mm-path-length were used. Measurements were made at temperatures in the range 15–20°C. RNA and subparticle solutions were dilute (25–50mg/litre). The measured $s_{20,w}$ approximates to $s^0_{20,w}$ at these concentrations.

MSE Mistral, MSE 18 and MSE Superspeed 65 centrifuges were used for preparative work.

### *Preparation and isolation of rabbit reticulocyte ribosomes, pH5 enzyme fraction and $(NH_4)_2SO_4$ fraction*

Rabbit reticulocytes were prepared and lysed as previously described (Arnstein *et al.*, 1964). As far as possible all subsequent operations were carried out at 0–4°C. The pellet obtained by centrifugation of the postmitochondrial supernatant at 105000$g_{av.}$ (MSE 8×25 rotor) for 60min was the polyribosome fraction. The pH5 enzyme fraction was prepared after the removal of ribosomes from the post-polyribosomal supernatant at 105000$g_{av.}$ for 1h as described by Arnstein *et al.* (1964). The $(NH_4)_2SO_4$ fraction containing elongation factors was isolated by the procedure of Hardesty *et al.* (1971).

### *Isolation of subribosomal particles*

*Reticulocyte subparticles.* The polyribosome fraction (100–200mg) was suspended in buffer (0.3M-KCl/3mM-$MgCl_2$/1mM-dithiothreitol/0.02M-Tris/HCl, pH7.6), made up to 20ml with the same buffer and at the same time adjusted to 0.5M-KCl. GTP (2.4mg; final concn. 0.2mM) and puromycin (2.75mg; 0.2mM) were added and the solution was kept at 37°C for 30min. The solution was then made 5% (w/v) in sucrose, cooled in an ice bath and layered on a 15–45% (w/v) sucrose gradient in overlay buffer (0.3M-KCl/3mM-$MgCl_2$/1mM-dithiothreitol/0.02M-Tris/HCl, pH7.6) in a B XIV rotor (capacity 675ml). The sample and overlay buffer displaced 250ml of 45% sucrose solution. Centrifuging was for 3h at 43000rev./min (210000$g_{av.}$; $r_{av.}$ 5.6cm) at approx. 5°C. The gradient was pumped out in 4×40ml, followed by 38×15ml fractions. Two well-separated components were obtained. The peak fractions were combined and the subparticles were obtained by centrifuging for 17h at 40000rev./min (MSE 8×25 rotor). The pellets were resuspended in 0.1M-$NH_4Cl$/2mM-$MgCl_2$/15% (v/v) glycerol/1mM-dithiothreitol/0.02M-Tris/HCl, pH 7.6.

*E. coli subparticles.* Frozen cells (*E. coli* M.R.E. 600) were obtained from the Microbiological Research Establishment, Porton, Wilts., U.K. Cells were disrupted by grinding with alumina, the ribosome fraction was obtained as described by Godson & Cox (1970) and subparticles were separated by zonal centrifugation (Cox & Pratt, 1973). A portion (10ml) of the ribosome solution (approx. 20mg/ml) was loaded on a gradient of 15–40% sucrose containing 0.06M-KCl/0.1mM-$MgCl_2$/0.01M-Tris/HCl, pH 7.6. The sample and overlay displaced 270ml of sucrose from the rotor. After centrifuging at 43000 rev./min for 3.5h at approx. 5°C (a titanium MSE B XIV rotor and MSE Superspeed 65 ultracentrifuge were used) fractions (4×50ml and then 35×15ml) were collected from the centre. To the fractions containing subribosomal particles, ethanol (2vol.) was added and the precipitate obtained after 30min at −12°C separated by centrifuging at 2500rev./min for 20min (1500*g*/min) and resuspended in 0.1mM-magnesium acetate/0.01M-Tris/HCl, pH7.6. The smaller subparticles were further purified by centrifuging for 3.5h at 43000rev./min (MSE B XIV rotor) through a second gradient (15–40% sucrose/0.01M-$MgCl_2$/0.05M-KCl/0.025M-Tris/HCl, pH7.6). Fractions were collected and the subparticles were recovered as described above.

*Isolation of rRNA*

rRNA was isolated from subribosomal particles by precipitation as the guanidinium salt as described by Cox (1968).

*Amino acid incorporation by the cell-free system*

The assay of the incorporation of L-[U-$^{14}$C]-phenylalanine into protein by ribosome fractions was based on the method described by Arnstein *et al.* (1964). Samples were diluted to approx. 0.2mg/ml in 'sample buffer' [medium $A_1$ of Arnstein *et al.* (1964), 0.25M - Sucrose / 25mM-KCl / 1mM - $MgCl_2$ / 1mM - dithiothreitol/50mM-Tris/HCl, pH7.6]. Each tube (0.5ml) contained approx. 10$\mu$g of L-subparticles, an excess (40$\mu$g) of S-subparticles, 25–50$\mu$g of pyruvate kinase, 5.0$\mu$mol of phosphoenolpyruvate, 0.123$\mu$mol of ATP and 31.5nmol of GTP, approx. 1mg of pH5 enzyme fraction, approx. 1mg of $(NH_4)_2SO_4$ fraction, 5$\mu$mol of neutralized GSH (reduced glutathione), 25$\mu$mol of KCl, 2.5$\mu$mol of $MgCl_2$, 12.5$\mu$mol of Tris/HCl, pH7.6, an unlabelled mixture of the 20 protein amino acids except phenylalanine (0.01$\mu$mol of each), [$^{14}$C]phenylalanine (The Radiochemical Centre, Amersham, Bucks., U.K.; sp. radioactivity 50mCi/mmol) and poly(U) (75$\mu$g), supplied by Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. The effective specific radioactivity was 30mCi/mmol after allowing for the endogenous amino acid pool. Polyphenylalanine synthesis was dependent on the $(NH_4)_2SO_4$ fraction (or on a 0.5M-KCl polyribosome wash fraction). Incubation was for 30min at 37°C. Isolation and filtration of the labelled protein was as described by Pratt & Cox (1971), but the radioactivity of the samples was measured by liquid-scintillation counting (Beckman LS 150 instrument; efficiency 80%); the scintillation fluid was toluene containing 5g of 2,5-diphenyloxazole and 0.25g of dimethyl-POPOP [1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene] per litre.

## Results

*Difference spectrophotometry*

*Effect of $Mg^{2+}$ on rRNA conformation in Nomura's (Traub & Nomura, 1969) reconstitution buffer.* Samples of rRNA in reconstitution buffer (Traub & Nomura, 1969) from which $Mg^{2+}$ was omitted (0.35M-KCl/10mM-Tris/HCl, pH7.2) were placed in both the sample and reference cells of the Cary 118 spectrophotometer. A syringe micro-burette was used to add increasing amounts of a concentrated solution of $MgCl_2$ to the sample cell and an equivalent volume of water was added to the reference cell. The difference spectrum observed was minimum at approx. 260nm and there was a small maximum at 290nm, but there was little or no change in $E_{280}$ (see Fig. 1). The difference spectrum obtained had the

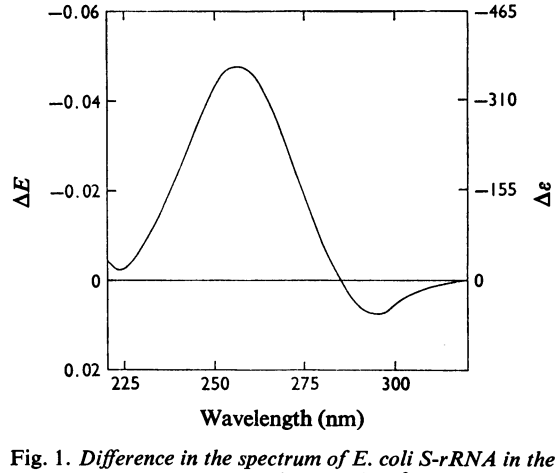


Fig. 1. *Difference in the spectrum of E. coli S-rRNA in the presence and absence of $Mg^{2+}$*

*E. coli* S-rRNA (43$\mu$g/ml) was dissolved in 0.17M-KCl/10mM-Tris/HCl, pH7.2. Samples (3ml) were placed in the sample and reference cells of a Cary 118 spectrophotometer. Then 20$\mu$l of 3M-$MgCl_2$ was added to the sample cell to give a final concentration of 20mM-$Mg^{2+}$, and 20$\mu$l of water was added to the reference cell. The difference in the spectrum of the two samples was measured before and after the additions were made. The net difference due to the addition of $Mg^{2+}$ is given in the Figure.

Table 1. *Values of K, n and* $\Delta\varepsilon^{\infty}_{260}$ *(eqn. 9) estimated for the titration of rRNA with* $Mg^{2+}$

The rRNA was extracted from the isolated subparticles as described in the Methods section, and dissolved in 10mM-Tris/HCl, pH7.2 containing the given concentration of KCl. The difference spectrum caused by the addition of $MgCl_2$ was measured and the change in absorbance at 260nm was used to estimate the equilibrium constant, $K_1$, and $n$ (see eqn. 9 and Figs. 2 and 3).

| Species | [KCl] (M) | $K_1$ | $n$ | $\Delta\varepsilon^{\infty}_{260}$ |
|---|---|---|---|---|
| *E. coli* S | 0.17 | 0.86±0.06 | 0.73±0.1 | 420 |
| *E. coli* S | 0.35 | 0.38±0.01 | 1.06±0.1 | 360 |
| *E. coli* S | 0.60 | 0.15±0.01 | 1.09±0.1 | 200 |
| *E. coli* S | 1.2 | 0.05±0.015 | 1.2±0.2 | 130 |
| *E. coli* L* | nil | 14±5 | 0.92±0.2 | 450 |
| *E. coli* L | 0.030 | 10±1 | 1.0±0.1 | 300 |
| *E. coli* L | 0.090 | 2.30±0.3 | 1.2±0.05 | 260 |
| *E. coli* L† | 0.35 | 0.245±0.01 | 1.16±0.05 | 260 |
| *E. coli* L | 0.60 | 0.190±0.03 | 1.1±0.1 | 190 |
| *E. coli* L | 1.2 | 0.102±0.03 | 1.12±0.2 | 120 |
| *E. coli* L‡ | 0–0.4 | — | — | 110 |
| Rabbit S | 0.35 | 0.40±0.02 | 0.99±0.1 | 210 |
| Rabbit L | 0.35 | 0.28±0.02 | 0.85±0.1 | 230 |

* The KCl-free solution was first made 20mM in $MgCl_2$ and $\varepsilon_{260}$ decreased by approx. 450; the addition of KCl then had no detectable effect on the spectrum ($\Delta\varepsilon_{260}< -10$).

† When $Sr^{2+}$ was used in place of $Mg^{2+}$ the values obtained were $n = 1.10$, $K_1 = 0.30$, $\Delta\varepsilon^{\infty}_{260} = 284$.

‡ KCl was added to L-rRNA in 0.01M-Tris/HCl, pH7.6; $\varepsilon_{260}$ decreased by 109 when the concentration of KCl was increased from 0 to 0.4M.

form expected if the addition of $Mg^{2+}$ led to the formation of A·U base-pairs (Cox, 1971). For S-rRNA of *E. coli* the maximum change in the spectrum that was found corresponded to a net increase of approx. 58 A·U base-pairs per molecule. This calculation is based on a value of 4500litre·$mol^{-1}$·$cm^{-1}$ for the change in $\varepsilon_{260}$ (the $E_{260}$/mol of nucleotide) on the formation of an A·U base-pair (Cox, 1971), and $\varepsilon_{260}$ 7750litre·$mol^{-1}$·$cm^{-1}$ for rRNA (Cox, 1970) and a molecular mass of approx. 560000 daltons (Loening, 1968). *E. coli* L-rRNA responded similarly to $Mg^{2+}$ concentration. A similar effect was seen on the spectrum of L-rRNA when $Sr^{2+}$ was used instead of $Mg^{2+}$ (see Table 1). When rabbit S-rRNA and L-rRNA were titrated with $Mg^{2+}$, the absolute changes in absorbance per nucleotide were smaller than those noticed for *E. coli* rRNA (see Fig. 2*a*), but the difference spectra were characteristic of the net formation of bihelical structures very rich in A·U base-pairs (i.e. $\Delta\varepsilon_{260} \gg \Delta\varepsilon_{280}$).

According to the model outlined in the Theoretical section, the equilibrium constant, $K_1$, and $n$, the number of $Mg^{2+}$ ions bound per nucleotide, may be calculated by use of eqn. (9). The double-logarithmic plot for *E. coli* L-rRNA (Fig. 3) is best fitted by a line in which $K_1$ has a value of 0.245±0.01 and $n$ approximates to unity ($n = 1.16 \pm 0.05$). Similar values are found for the other rRNA species studied (Table 1).

Assuming that $n = 1$, then eqn. (8) simplifies to:

$$\frac{\Delta\varepsilon^{\infty}_{\lambda}}{\Delta\varepsilon_{\lambda}} - 1 = \frac{1}{K_1[Mg^{2+}]} \qquad (16)$$

This allows $K_1$ to be estimated rapidly as the reciprocal of $[Mg^{2+}]_{0.5}$, the concentration of $Mg^{2+}$ at which $\Delta\varepsilon_{\lambda} = 0.5\Delta\varepsilon^{\infty}_{\lambda}$.

*Effect of* $Mg^{2+}$ *on rRNA at different concentrations of KCl.* The secondary structure of *E. coli* L-rRNA is already largely formed in 0.01M-Tris/HCl, pH7.6 at 25°C. The addition of KCl up to 0.4M had little further effect, since $\varepsilon_{260}$ decreased by approx. 100litre·$mol^{-1}$·$cm^{-1}$ (see Table 1). A much larger effect was found when $MgCl_2$ (4mM) was added to L-rRNA in 0.01M-Tris/HCl, pH7.6, when $\varepsilon_{260}$ decreased by approx. 450litre·$mol^{-1}$·$cm^{-1}$; the further addition of 0.4M-KCl did not affect the spectrum (see Table 1).

The difference spectra that were observed on titration of *E. coli* S-rRNA (see Fig. 2*b* and Table 1) and *E. coli* L-rRNA (see Table 1) with $MgCl_2$ were measured at concentrations of KCl up to 1.2M. The addition of $MgCl_2$ to 1.2M-KCl solutions led to a decrease in $\varepsilon_{260}$ of rRNA indicating that this high concentration of KCl was not equivalent to 20mM-$MgCl_2$ in its effect on the rRNA spectrum. The difference spectra had the form illustrated in Fig. 1 but the magnitude of the difference was less, as might be expected (see eqn. 15 and those following) if increasing the concentration of KCl had an effect on $\varepsilon^{0}_{\lambda}$ which would lead to a decrease in $\Delta\varepsilon^{\infty}_{\lambda} = \varepsilon^{\infty}_{\lambda} - \varepsilon^{0}_{\lambda}$.

Over the range of KCl concentrations studied, the effects of $Mg^{2+}$ on the spectrum of rRNA were described by eqn. (11) (see Fig. 2 and Table 1). An estimate of $m$, the number of $K^+$ ions that were displaced per nucleotide on the addition of $MgCl_2$, is obtained from eqn. 13 by a double-logarithmic plot of [KCl] against $K_1$ (Fig. 4). This shows that $m$ is approx. 1.3; assuming a value for $n$ of unity (see above), this means that, overall, slightly more than one $K^+$ ion is displaced by one $Mg^{2+}$ ion per nucleotide. The value of $K_{10}$, the overall equilibrium constant for the formation of $N_{II}$ from $N_I$ in the presence of $K^+$ and $Mg^{2+}$, was estimated to be 80±5 (Fig. 4) so that a ratio of 1 $Mg^{2+}$ ion:40 $K^+$ ions is sufficient to allow 67% of the $Mg^{2+}$-sensitive conformation to form. The ratio of the two ions may reflect the ability of $Mg^{2+}$ to compete with $K^+$ for diesterified phosphate residues.

There are two ways in which $Mg^{2+}$ ions may act as described by eqn. (15). First, $Mg^{2+}$ may induce the same conformation as KCl but more efficiently, and secondly, $Mg^{2+}$ may induce structures that are specific to a narrow range of bivalent cations. The

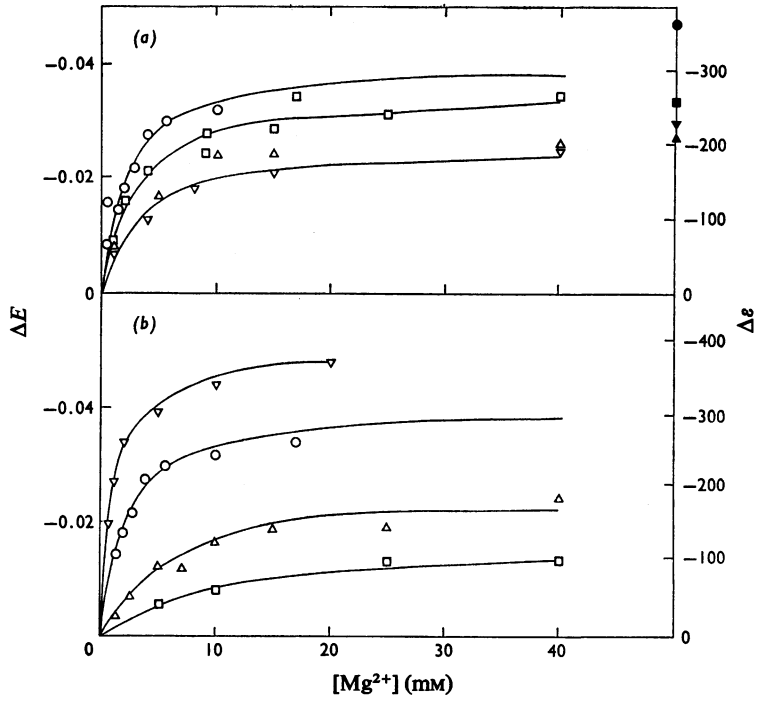


Fig. 2. *Effect of $Mg^{2+}$ on the $E_{260}$ of rRNA*

(*a*) The solvent was 0.35M-KCl/10mM-Tris/HCl, pH7.2, and the change in the spectrum on the addition of $MgCl_2$ was measured as described in the legend to Fig. 1. In each case a difference spectrum having the same features as that given in Fig. 1 was noticed, and the $\Delta E_{260}$ was calculated. The symbols indicate measured values and in each case the full line is a theoretical curve calculated by means of eqn. (9) and by using the constants given in Table 1. ○, *E. coli* S-rRNA; □, *E. coli* L-rRNA; △, rabbit S-rRNA; ▽, rabbit L-rRNA. The solid symbols show the estimated value of $\Delta\varepsilon^{\infty}$ (see Table 1). (*b*) *E. coli* S-rRNA was titrated with $MgCl_2$ at different concentrations of KCl (0.17–1.2M-KCl/0.01M-Tris/HCl, pH7.2) as described in (*a*). As in (*a*) the symbols indicate measured values and the line denotes theoretical values calculated by means of eqn. (9) by using the data of Table 1. ▽, 0.17M-KCl; ○, 0.35M-KCl; △, 0.6M-KCl; □, 1.2M-KCl.

results allow both possibilities. In addition to effects that are detectable by our spectrophotometric method, $Mg^{2+}$ ions may influence rRNA conformation in other ways. We would expect such changes, if any, to obey eqn. (10), because an important step is probably the neutralization of the diesterified phosphate residues of the ribose–phosphate backbone.

*Difference spectra at elevated temperatures.* $Mg^{2+}$ was added to the sample cell at 20°C, and the difference spectrum was measured as described above. The temperature of both sample and reference cells were increased to the same value and the difference spectrum was again measured. In this way a series of difference spectra of rRNA in KCl solution with and without $Mg^{2+}$ (see Fig. 5) were obtained at a range of elevated temperatures. The difference in the extinction of the $Mg^{2+}$-containing and $Mg^{2+}$-free solutions of rRNA increased as the temperature was raised (see Fig. 5). It was inferred from these difference spectra that at the elevated temperatures, as well as at 20°C, the presence of $MgCl_2$ led to a net increase of mainly A·U base-pairs. The stabilizing effect of $Mg^{2+}$ was more pronounced at 37°C (a temperature favoured for reconstitution experiments; e.g. see Mizushima & Nomura, 1970) than at 20°C.

*Effect of $Mg^{2+}$ on the thermal stability of rRNA conformation ('melting' curves).* That $Mg^{2+}$ stabilized rRNA secondary structure against thermal denaturation was shown when the 'melting' profile was measured over the range 25–95°C. In these experiments the sample and reference cells both contained samples of the same solution. The reference cell was kept at 25°C throughout while the sample cell was heated; the difference in the spectrum of the two samples was then measured over the range 220–330nm. This procedure was used to measure the thermal stability of the secondary structure of *E. coli* S-rRNA and L-rRNA and of rabbit S-rRNA and L-rRNA in the solvents 0.35M-KCl/0.01M-Tris/HCl,

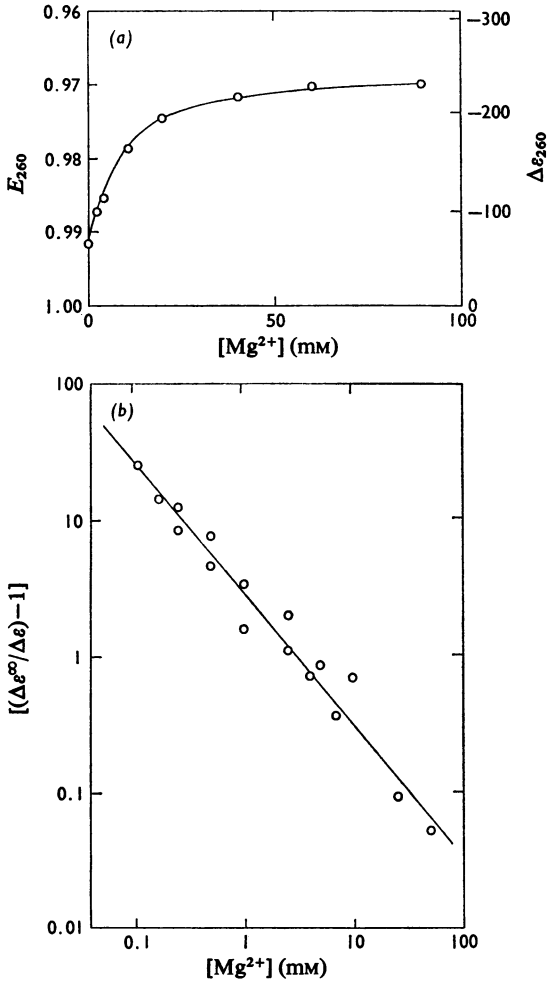


Fig. 3. *Estimation of the constant $K_1$ (see eqn. 1) for the reaction of $Mg^{2+}$ with E. coli L-rRNA*

(*a*) *E. coli* L-rRNA (43 $\mu$g/ml) in a 0.35 M-KCl/0.01 M-Tris/HCl, pH 7.2, was titrated with $MgCl_2$ and $\varepsilon_{260}$ was measured as a function of $MgCl_2$ concentration. (*b*) The values of $\varepsilon_{260}$ given in (*a*) were used to calculate $[(\Delta\varepsilon^{\infty}/\Delta\varepsilon)-1]$ as a function of $MgCl_2$ concentration. The intercept at 1 mM-$MgCl_2$ on a double-logarithmic plot (see eqn. 9) provides an estimate of $K_1$ ($K_1 = 0.245 \pm 0.010$), and the slope is a measure of $n$ ($n = 1.16 \pm 0.10$) in eqn. (1). The error in $(\Delta\varepsilon^{\infty}/\Delta\varepsilon)-1$ for a 1% error in $\Delta\varepsilon^{\infty}$ or in $\Delta\varepsilon$ is within the diameter of the symbol.

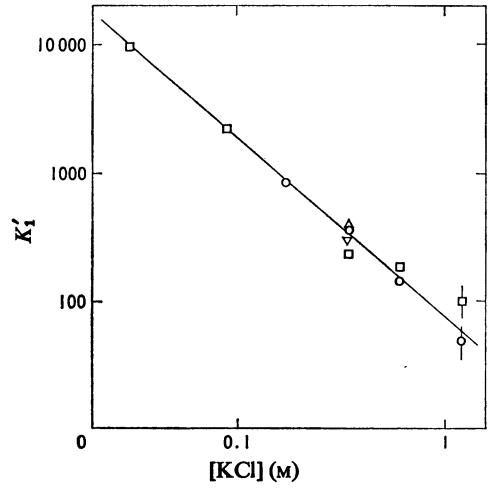


Fig. 4. *Dependence of $K_1$ on KCl concentration and the evaluation of $K_{10}$ and n (see eqn. 10)*

The values of $K_1$ given in Table 1 that fit the data of Fig. 2(*b*) were used in a double-logarithmic plot of $K_1'$ ($K_1' = K_1 \times 10^{-3}$; $K_{10}$ has the dimensions $mol^{1.3}/mol$) against [KCl] (see eqn. 13). The gradient is $m = 1.3 \pm 0.1$ and the intercept at 1 M-KCl is $K_{10} = 80 \pm 5$. ○, *E. coli* S-rRNA; □, *E. coli* L-rRNA; △, rabbit S-rRNA; ▽, rabbit L-rRNA. The bar indicates the error in $K_1$ obtained from plots such as that given in Fig. 3(*b*).

pH 7.2, and 20 mM-$MgCl_2$/0.35 M-KCl/0.01 M-Tris/HCl, pH 7.2. The results are summarized by the plot of the increments in $E_{260}$ and $E_{280}$ against temperature in Figs. 6(*a*)–6(*d*). In each case the effect of $Mg^{2+}$ was to increase the thermal stability of secondary structure by about 5°C, since, compared with $Mg^{2+}$-free-rRNA, there was a shift in the 'melting' profile by about 5°C towards higher temperatures when $Mg^{2+}$ was present. In addition, the early part of the profile was slightly steeper in the presence than in the absence of $Mg^{2+}$, as though the denaturation process was more co-operative (cf. the 'melting' of the functional subparticles; Cox *et al.*, 1973).

*Effect of $Mg^{2+}$ on the c.d. of rRNA.* The measurements of c.d. lacked the accuracy of the u.v. absorbance measurements because a single-beam instrument was used. Careful and repeated measurements on the same and on different samples showed that the addition of $Mg^{2+}$ increased c.d., and the c.d. difference spectra that were obtained for the four rRNA species were consistent with the formation of additional A·U base-pairs (Fig. 7) [see Cox *et al.* (1976*a*) for discussion of the c.d. of rRNA] that increase the extent of base-pairing by approx. 10%.

*Effect of $Mg^{2+}$ on $s_{20,w}$ of E. coli rRNA*

The sedimentation profiles of dilute solutions (approx. 50 mg/litre) of *E. coli* S-rRNA and L-rRNA were measured at 10–17°C. *E. coli* S-rRNA sedimented largely as a single boundary with less than 20% of dimer ($s_{20,w}$ approx. 23 S) and the addition of $MgCl_2$ led to an increase in $s_{20,w}$ (see Fig. 8), but no

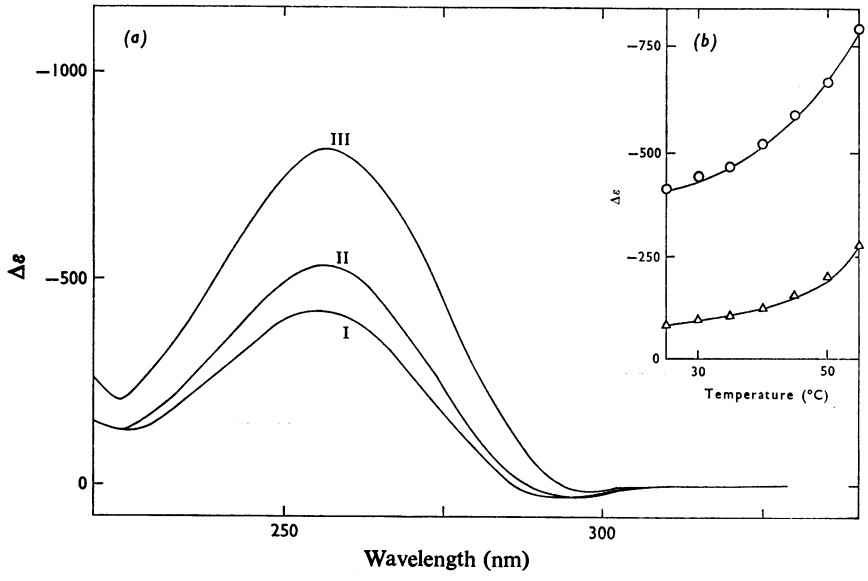


Fig. 5. *Difference in the spectrum of E. coli S-rRNA in the presence and absence of* $Mg^{2+}$ (20mM) *at temperatures in the range* 25–55°C

The solvent was 0.35M-KCl/0.01M-Tris/HCl, pH7.2; 3M-$MgCl_2$ (20$\mu$l) was added to the sample (3ml) in the sample cell (final concn. 20mM) and water (20$\mu$l) was added to the reference cell. The difference in the spectrum was measured before and after the addition of $Mg^{2+}$. The temperature of both sample and reference solutions (which was controlled by a Haake circulating-water bath) was then increased by 5°C and the difference spectrum was again measured. Further measurements were made at 5°C intervals up to 55°C. At 20°C, $E_{260} = 1.0$ for the $Mg^{2+}$-free solution. (*a*) Difference spectra: I, 25°C; II, 40°C; III, 55°C. (*b*) (inset) ○, dependence of $\Delta\varepsilon_{260}$ [$\varepsilon_{260}$ (20mM-$MgCl_2$)−$\varepsilon_{260}$ ($Mg^{2+}$-free)], and △, dependence of $\Delta\varepsilon_{280}$ [$\varepsilon_{280}$ (20mM-$MgCl_2$)−$\varepsilon_{280}$ ($Mg^{2+}$-free)] on temperature. At a particular temperature, $\Delta\varepsilon_{260}/\Delta\varepsilon_{280}$, measures the (A+U)/(G+C) ratio of additional bihelical structure present (Cox, 1971) in 20mM-$MgCl_2$.

change in the proportion of monomer was noticed. The increase in $s_{20,w}$ on increasing the concentration of $MgCl_2$ accords with the work of Möller & Boedtker, (1961) which showed that in 0.15M-KCl the $s_{20,w}$ of *E. coli* S-rRNA increased as the concentration of $Mg^{2+}$ increased. Whereas the changes in extinction and in c.d. were largely complete at 10mM-$MgCl_2$, the $s_{20,w}$ value continued to increase as $Mg^{2+}$ concentration increased. Thus the increase in $s_{20,w}$ is attributable partly to the formation of additional intramolecular bonds and partly to a contraction in hydrodynamic volume arising from a more effective screening of charged phosphate residues. The hydrodynamic volume would be expected to decrease until the point is reached where the polymer dimensions are unperturbed by solvent–polymer interactions (the $\theta$-point of Flory; see Flory, 1953). At 25°C in 0.35M-KCl/10mM-Tris/HCl, pH7.2, the point is approached at approx. 90mM-$MgCl_2$, since rRNA was found to precipitate when the concentration of $MgCl_2$ was increased further. Thus the $s_{20,w}$ might be expected to continue to increase until 90mM-$MgCl_2$ is attained. It is clear that the $Mg^{2+}$ leads to a compact particle; indeed, with *E. coli* S-rRNA ($s_{20,w}$ = 21S in 15mM-$MgCl_2$; 24S in 40mM-$MgCl_2$) increasing the $Mg^{2+}$ concentration brings $s_{20,w}$ much closer to the value (31S) found for the native subparticle in the same conditions.

*Extension to rabbit S- and L-subparticles*

The effect of $Mg^{2+}$ on rabbit subribosomal particles was studied by measuring their capacity to function in poly(U)-directed polyphenylalanine synthesis in a cell-free system after they were exposed to particular concentrations of $NH_4Cl$ and $MgCl_2$. For practical purposes $NH_4Cl$ was preferred to KCl, and both salts appear to be equivalent in their effect on L-subparticles (Weiss *et al.*, 1973). The first type of experiment (see Fig. 9) was designed to establish the concentration of $Mg^{2+}$ needed to stabilize L-subparticles against salt-shock treatment with particular concentrations of $NH_4Cl$. The activity of L-subparticles in polyphenylalanine synthesis was measured after treatment with the required $NH_4Cl$ concentration at 0°C for 2h but with various concentrations of

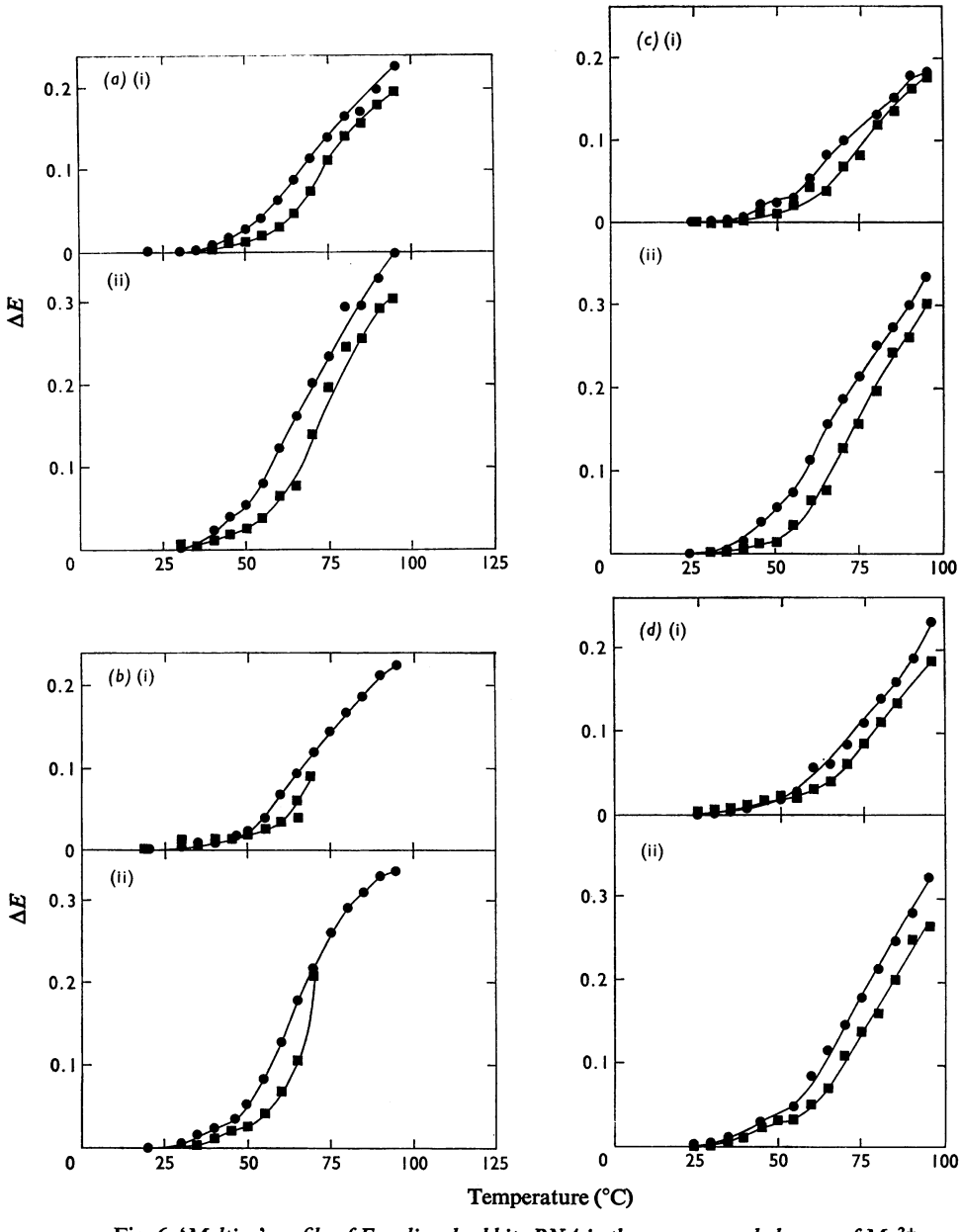


Fig. 6. *'Melting' profile of E. coli and rabbit rRNA in the presence and absence of* $Mg^{2+}$

The difference in the spectrum of a heated sample and a sample kept at 20°C was measured. The difference in the spectrum was measured at 5°C intervals. The increments in $E_{280}$ (part i) and in $E_{260}$ (part ii) were calculated and corrected for changes in extinction due to changes in rRNA concentration on heating arising from the thermal expansion of water. ●, Solvent was 0.35M-KCl/10mM-Tris/HCl, pH7.2; ■, solvent was 0.35M-KCl/20mM-$MgCl_2$/10mM-Tris/HCl, pH7.2. In each case $E_{260}$ = 1.0 at 20°C. (*a*) *E. coli* S-rRNA; (*b*) *E. coli* L-rRNA; (*c*) rabbit S-rRNA; (*d*) rabbit L-rRNA.

added $MgCl_2$ (see Fig. 9). The results provide an estimate of $[Mg^{2+}]_{0.5}$ at that particular $NH_4Cl$ concentration. The assay measures irreversible changes in ribosome function, and a change induced by the $NH_4Cl/MgCl_2$ solvent that was reversed during the assay for polyphenylalanine synthesis would not

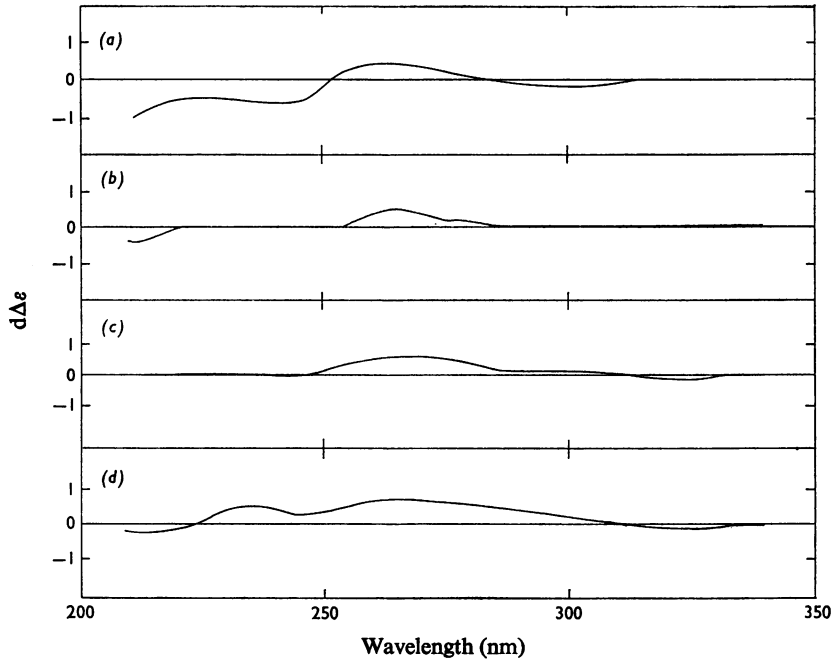


Fig. 7. *Effect of $Mg^{2+}$ on the c.d. spectrum of rRNA*

The rRNA was dissolved on 0.35M-KCl/10mM-Tris/HCl, pH7.2, to give an $E_{260}$ of not greater than 1.2. A 3ml sample was placed in a 1cm-path-length cell, 20$\mu$l water was added and the $E_{260}$ measured accurately before measuring the c.d. spectrum in a Rousset–Jouan Dichrograph, model CD185. The c.d. of a similar 3ml sample to which 20$\mu$l of 3M-$MgCl_2$ had been added was also measured, and the difference between the two samples was obtained. The curves shown are the mean values of about five independent experiments. (*a*) *E. coli* S-rRNA; (*b*) *E. coli* L-rRNA; (*c*) rabbit reticulocyte S-rRNA; (*d*) rabbit reticulocyte L-rRNA.

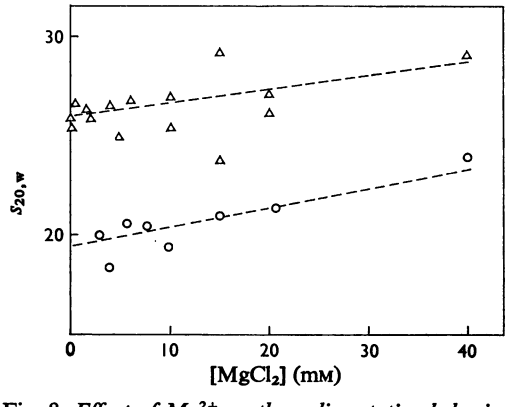


Fig. 8. *Effect of $Mg^{2+}$ on the sedimentation behaviour of E. coli L- and S-rRNA*

The two rRNA species were extracted from the isolated subparticles. rRNA was dissolved in 0.35M-KCl/10mM-Tris/HCl, pH7.2 ($E_{260} = 0.7$). Sufficient 3M-$MgCl_2$ was added to give the $Mg^{2+}$ concentration required, and the sedimentation coefficient measured at 5–10°C in a Beckman model E analytical ultracentrifuge by using the u.v. optical system. △, L-rRNA; ○, S-rRNA.

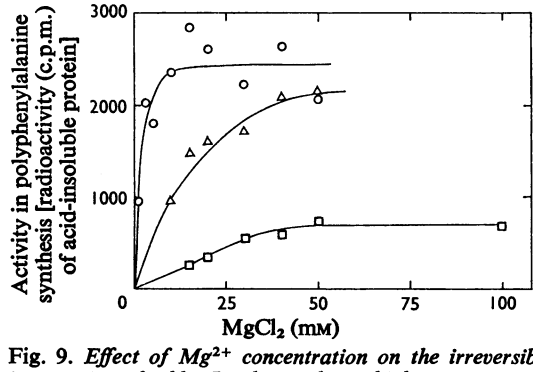


Fig. 9. *Effect of $Mg^{2+}$ concentration on the irreversible inactivation of rabbit L-subparticles at high concentrations of $NH_4Cl$*

Rabbit L-subparticles (3mg/ml) were kept at 0°C for 2h at different concentrations of $NH_4Cl$ (○, 0.5M; △, 1.4M; □, 2.1M) and $MgCl_2$, all in 10mM-Tris/HCl, pH7.6. After this salt-shock treatment the solutions were diluted to 0.2mg of L-subparticles/ml with 0.025M-KCl/1mM-$MgCl_2$/0.05M-Tris/HCl, pH7.6, and assayed at 37°C in the standard cell-free system for poly(U)-directed polyphenylalanine synthesis (Arnstein *et al.*, 1964).

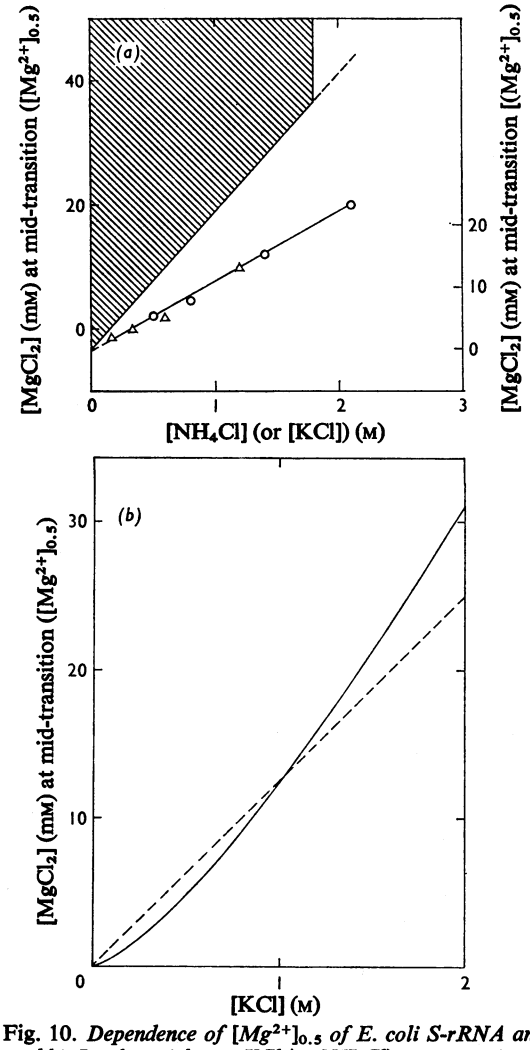


Fig. 10. *Dependence of $[Mg^{2+}]_{0.5}$ of E. coli S-rRNA and rabbit L-subparticles on KCl (or $NH_4Cl$) concentrations*

(*a*) Values of $[Mg^{2+}]_{0.5}$ (see eqn. 14) were obtained from Fig. 2(*b*) for *E. coli* rRNA and from Fig. 9 for rabbit L-subparticles. The $Mg^{2+}$ concentration of L-subparticle solutions was larger by about 1 mM than that of added $MgCl_2$ because of the addition of $Mg^{2+}$ that is bound to active L-subparticles (e.g. Petermann & Pavlovec, 1967; Weiss & Morris, 1973). The slope of the plot of $[Mg^{2+}]_{0.5}$ against [KCl] for *E. coli* S-rRNA and for the plot of $[Mg^{2+}]_{0.5}$ against $[NH_4Cl]$ for rabbit L-subparticles both correspond to 1 mM-$MgCl_2$/80 mM-KCl (or $NH_4Cl$). This value corresponds to $K_{10}$ in eqn. (10). The shaded portion indicates the conditions of $[Mg^{2+}]$ and [KCl] or $[NH_4Cl]$ where rabbit L-subparticles would be expected to retain 67% or more of full activity. △, Data for *E. coli* S-rRNA; ○, data for rabbit L-subparticles. (*b*) ——, Dependence of $[Mg^{2+}]_{0.5}$ on KCl concentration was calculated by using eqn. (14) when $m = 1.3$ (see Fig. 4) and $n = 1.0$ (see Table 1 and also Fig. 3). ----, Dependence of $[Mg^{2+}]_{0.5}$ on KCl concentrations on the basis of the assumption that $m = n = 1$.

have been detected. This experimental design was necessary because withdrawing $Mg^{2+}$ from ribosomes, e.g. by chelation with EDTA, destroys them (Gould *et al.*, 1966), so that adding $MgCl_2$ to $Mg^{2+}$-free ribosomes was not feasible. The results (see Fig. 9) showed that $[Mg^{2+}]_{0.5}$ increased as the concentration of $NH_4Cl$ increased. The values of $[Mg^{2+}]_{0.5}$ that were obtained yielded a straight line of slope 1 $Mg^{2+}$ ion/80 $NH_4^+$ ions, when plotted against $NH_4Cl$ concentration (Fig. 10). The intercept on the $[Mg^{2+}]_{0.5}$ axis was negative on extrapolation to zero $NH_4Cl$ concentration, possibly because the contribution of $Mg^{2+}$ tightly bound to L-subparticles was neglected, so that the true concentration of $Mg^{2+}$ was underestimated. Active L-subparticles need a concentration of bound $Mg^{2+}$ (approx. 0.2 mol of $Mg^{2+}$/mol of RNA-*P*) to maintain them in a functional state (Petermann & Pavlovec, 1967; Weiss *et al.*, 1973), so that they are already partly in the form of $Mg^{2+}$ salt and contribute approx. 0.36 $\mu$mol of $Mg^{2+}$/mg of L-subparticles. The final concentration was 3 mg of L-subparticles/ml, so that the contribution of the ribosome fraction was approx. 1 $\mu$mol/ml. Thus the $Mg^{2+}$ concentration calculated on the basis of the contribution of the buffer alone (see Fig. 10) should be increased by approx. 1 mM to reflect the true $Mg^{2+}$ concentration. Values of $[Mg^{2+}]_{0.5}$ for the effect of $MgCl_2$ on $\varepsilon_{260}$ of rRNA at different concentrations of KCl (see Fig. 2) are also given in Fig. 10(*a*). The slope measures $K_{10}$ (see eqn. 14) provided that $m \simeq n \simeq 1$, and this appears to be a useful approximation over the range 0–2 M-KCl (or $NH_4Cl$), as is shown in Fig. 10(*b*). The best-fit straight line was a gradient of 1 $Mg^{2+}$ ion/80 $K^+$ ions, the same as for the L-subparticles. We conclude that the values of $K_{10}$ for rRNA and for rabbit L-subparticles are very similar.

Although two different assays were used, the data given in Figs. 2 and 9 and summarized in Fig. 10 are consistent with the notion that $Mg^{2+}$ is needed by ribosomes to maintain a particular (native) conformation of the rRNA moiety (cf. Edelman *et al.*, 1960; Cox *et al.*, 1976*b*); and that dependence of RNA conformation on $Mg^{2+}$ concentration is quantitatively the same for L-subparticles as for isolated L-rRNA.

### *Stability of rabbit S- and L-subparticles at high concentrations of $NH_4Cl$ with $MgCl_2$ kept at 1 $Mg^{2+}$/40 $NH_4^+$*

The inference from Fig. 10 is that 67% of full activity of rabbit L-subparticles is retained at an $Mg^{2+}/NH_4^+$ ratio of 1:40. The stability of ribosomes was studied further by examining the effect of $NH_4Cl$ on ribosomes when the $Mg^{2+}$ concentration was maintained at 1 mol of $Mg^{2+}$/40 mol of $NH_4^+$ (i.e. $[Mg^{2+}] \simeq 2[Mg^{2+}]_{0.5}$). The results (see Fig. 11) showed that rabbit S-subparticles were still 40%

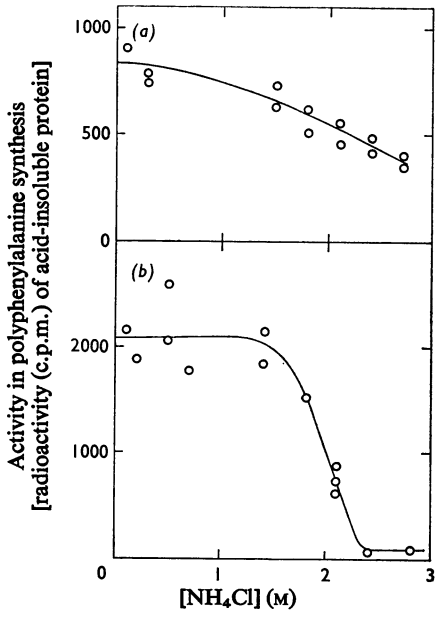


Fig. 11. *Irreversible inactivation of rabbit S-subparticles (a) and L-subparticles (b) by $NH_4Cl$ treatment, with sufficient $Mg^{2+}$ to maintain the 'native' rRNA concentration*

A solution of 4M-$NH_4Cl$/0.1M-$MgCl_2$/20mM-Tris/1mM-dithiothreitol / 15% (v/v) glycerol/HCl, pH7.6, was prepared and diluted with 15% (v/v) glycerol/1mM-dithiothreitol/20mM-Tris/HCl, pH7.6, to yield a solution of 3mg of S-subparticles (or L-subparticles)/ml of $NH_4Cl$ buffer (e.g. 0.21M, 0.7M, 1.4M, 2.1M and 2.8M-$NH_4Cl$, and $MgCl_2$ in the ratio 1 $Mg^{2+}$ ion:40 $NH_4^+$ ions). The solution was kept at 0°C for 2h, then diluted to 0.2mg of S-subparticles (or of L-subparticles)/ml with 0.25M-sucrose / 0.025M-KCl/1mM-$MgCl_2$ / 0.05M-Tris / HCl, pH7.6, and assayed in a cell-free system for poly(U)-directed polyphenylalanine synthesis.

active in polyphenylalanine synthesis after exposure to 2.8M-$NH_4Cl$/70mM-$MgCl_2$/0.02M-Tris/HCl, pH 7.6. The results are noteworthy because they show the stability of rabbit ribosomes to $NH_4Cl$ when $Mg^{2+}$ is not limiting. We infer that the native structure of the rRNA moiety is largely maintained during the salt treatment. The inactivation of L-subparticles over a narrow range of $NH_4Cl$ concentration needs explanation. The formation of a well-defined split-protein fraction comprising about eight proteins (approx. 20% of the protein moiety) and a core particle under these conditions was subsequently shown, and active subparticles were reconstructed from these fractions [see the next-but-one paper following (Cox & Greenwell, 1976)].

Table 2. *Estimate of the net increase in A·U base-pairs per molecule on addition of $Mg^{2+}$ to rRNA in* 0.35M-*KCl/* 10mM-*Tris/HCl*, pH7.2

The observations given in Fig. 2 were used to calculate the net increase in A·U base-pairs at the highest $Mg^{2+}$ concentration used (6–40mM). It was assumed that the $\varepsilon_p$ of rRNA in the native conformation is 7750litre·$mol^{-1}$·$cm^{-1}$ and the change in $\varepsilon_p$ on forming A·U base-pairs is 4500litre·$mol^{-1}$·$cm^{-1}$ at 260nm. ($\varepsilon_p$ is the molar extinction coefficient per g-atom of phosphorus in an RNA species.) Values for molecular weights are taken from Loening (1968).

| RNA species | $10^{-6}$ × Mol.wt. | Maximum observed $\Delta\varepsilon_{260}$ (see Fig. 2a) | Net increase in A·U base-pairs per molecule |
|---|---|---|---|
| *E. coli* S | 0.56 | 310 | 58 |
| *E. coli* L | 1.07 | 265 | 95 |
| Rabbit S | 0.70 | 180 | 42 |
| Rabbit L | 1.72 | 191 | 110 |

## Discussion

The aim of the present work was to investigate the effect of $Mg^{2+}$ on rRNA conformation and to relate it to the $Mg^{2+}$ requirement of ribosomes. The results show that $Mg^{2+}$ induces changes in the conformation of rRNA even in the presence of high concentrations of univalent cation. The changes noticed on the addition of $Mg^{2+}$ were a net increase in A·U base-pairs (see Table 2) shown by both c.d. and u.v. measurements, and an increase in the thermal stability of bihelical secondary structure. In the absence of $Mg^{2+}$, rRNA has a highly ordered secondary structure as revealed by an analysis of optical properties (Cox, 1971; Cox *et al.*, 1976*a*). For *E. coli* S-rRNA, approx. 65% of the residues may form base-pairs and an additional approx. 20% of the residues contribute to hypochromism presumably because they 'stack' in single-stranded regions (Cox *et al.*, 1976*a*). Evidently $Mg^{2+}$ stabilizes further interactions between base residues probably by screening phosphate charges more efficiently, and the decrease of electrostatic repulsions between phosphate residues that results is seen as an increase in bihelix stability. Efficient charge neutralization in addition to formation of additional intramolecular bonds account for the increase in $s_{20,w}$ with increasing $Mg^{2+}$. A value of $n \simeq 1$ (see eqn. 10) might be expected where the phosphate residues of RNA are screened, e.g. by $K^+$, so that the charge on one residue has little effect on its neighbours, with the result that $Mg^{2+}$ may also interact with one residue without displacing a counter-ion from the next neighbour.

The suggestion that *E. coli* S-rRNA in reconstitution buffer, $Mg^{2+}$ allows a further approx. 58 A·U base-pairs/molecule to be formed may be examined in the light of knowledge of the primary sequence and secondary structure proposed by Ehresmann *et al.* (1975). Additional A·U base-pairs may be formed either by $Mg^{2+}$ stabilizing short or imperfect A·U helices within a hairpin loop or closing a hairpin loop or by $Mg^{2+}$ stabilizing the formation of A·U helices formed through the interaction of residues located on different (and more distant) partly bihelical hairpin loops. The proposal that additional A·U base-pairs are formed is open to further study, e.g. by chemical-modification experiments. The spectrophotometric measurements (both u.v. and c.d.) show a net change, and it is not possible to decide from them whether the addition of $Mg^{2+}$ leads to unfolding and rearrangement of previously formed hairpin loops or simply to the formation of additional base-pairs.

*Comparison with the $Mg^{2+}$ requirement for the reassembly of E. coli S-subparticles*

The reconstitution of *E. coli* S-subparticles from S-rRNA and proteins is optimum in 0.35M-KCl. The $Mg^{2+}$-dependence of reassembly in reconstitution buffer was examined by Traub & Nomura (1969), who used the capacity to function in polyphenylalanine synthesis as a measure of reassembly. About half the maximum activity was attained at approx. 5mM-$MgCl_2$ (cf. 3mM for the mid-point in the change in $\varepsilon_{260}$; see Fig. 2) and maximum activity was found at 10mM-$MgCl_2$ (1 $Mg^{2+}$ ion/35 $K^+$ ions; cf. 1 $Mg^{2+}$ ion/40 $K^+$ ions to effect 67% of the $Mg^{2+}$-sensitive conformational change in S-rRNA). The binding of individual proteins to *E. coli* S-rRNA is also optimum in 0.35M-KCl/10–20mM-$MgCl_2$. The notion that the $Mg^{2+}$ is needed to preserve the 'native' rRNA conformation is consistent with these results for S-subparticle re-assembly (cf. Zimmerman, 1974).

*Comparison with the $Mg^{2+}$ requirement of functional ribosomes*

Both the results and the simple theory are in accord with previous work on the binding of $Mg^{2+}$ by ribosomes. Edelman *et al.* (1960) and Petermann & Pavlovec (1967) showed that the amount of $Mg^{2+}$ bound to rat liver ribosomes was a function of both [$Mg^{2+}$] and the univalent cation concentration. Weiss *et al.* (1973) showed that the activity of *E. coli* S- and L-subparticles in KCl (or $NH_4Cl$)/$MgCl_2$ solutions was preserved provided that the amount of bound $Mg^{2+}$ was maintained at approx. 0.2mol of $Mg^{2+}$/mol of RNA phosphorus [cf. Cohn *et al.* (1969) who found 0.095 strong $Mn^{2+}$-binding sites/mol of *E. coli* rRNA phosphorus]. At lower values of bound $Mg^{2+}$ the subparticles were inactivated and became more sensitive to ribonuclease action. Weiss *et al.* (1973) also established that ribosomes have a specific need for certain bivalent cations (e.g. $Mg^{2+}$ or $Mn^{2+}$). Ghysen *et al.* (1970) measured the stability of *E. coli* subribosomal particles as a function $R$, the ratio of monovalent cations to $Mg^{2+}$, over the range 0–0.25M-KCl and 0–0.3M-Tris/acetate, and showed that stability was governed by $R$ rather than by the absolute $Mg^{2+}$ concentration. This is equivalent to the case where $m = n = 1$ in eqn. (11), which may then be written in terms of $R$ (eqn. 17).

$$K_{10} = \frac{[N_{II}]}{[N_I]} R \qquad (17)$$

It was shown above (see Fig. 10*b*) that although $m \simeq 1.3$, $n \simeq 1$, the approximation $m = n = 1$ is useful over the range 0–2M-KCl (or $NH_4Cl$) which covers the conditions used in the experiments of Ghysen *et al.* (1970). A value of $K_{10}$ could not be derived from the data of Ghysen *et al.* (1970), but an estimate of $K_{10}$ for rabbit L-subparticle was obtained from the data given in Fig. 9 which shows that $K_{10}$ for the subparticle is close to that found for rRNA (Fig. 4*a*). This finding and the previous work discussed above support the notion that $Mg^{2+}$ is needed by ribosomes to maintain a particular rRNA conformation. The possibility remains that $Mg^{2+}$ may also fulfil other functions in stabilizing ribosome structure. If the conformation of rRNA within the subparticle is regarded as 'native', it appears important that in studying the properties of the isolated molecule the concentration of $Mg^{2+}$ should be sufficient to maintain the 'native' structure both during the isolation of rRNA and in any subsequent procedures.

*Comparison with other studies of rRNA conformation*

$Mg^{2+}$ was shown to affect the mobility of rRNA on gel electrophoresis, e.g. Morris *et al.* (1975) found that S-rRNA and L-rRNA of *E. coli* could be resolved on gel electrophoresis into different cation-specific conformational forms. Restoring the $Mg^{2+}$ concentration alone was not sufficient to restore the original conformation; a heating step was also necessary, possibly indicating specific binding of $Mg^{2+}$.

Evidence for interactions between distant parts of rRNA brought about by $Mg^{2+}$ was obtained by Ungewickell *et al.* (1975) on the basis of partial nuclease digests. Both these observations show that $Mg^{2+}$ is important to induce particular rRNA conformations and they complement our present study.

*Comparison with the effect of $Mg^{2+}$ on tRNA conformation*

$Mg^{2+}$ is known to be necessary for tRNA function, and many studies of the effect of $Mg^{2+}$ on

tRNA conformation have been reported. Willick & Kay (1971) used c.d. measurements to probe the effect of $Mg^{2+}$ on tRNA conformation. The results were analysed by using a two-state model (cf. the Theoretical section) and the concentrations of $Mg^{2+}$ needed to reach the mid-point of the transition were comparable with the data given above. The mid-point of the c.d. change was 2.5mM-$Mg^{2+}$ when the solvent contained 0.19M-KCl (i.e. one $Mg^{2+}$ ion/80$K^+$ ions) and was about 25mM in the presence of 1M-KCl (1$Mg^{2+}$ ion/40$K^+$ ions). Willick & Kay (1971) estimated that about 3$Mg^{2+}$ ions were bound per tRNA molecule (approx. 4$Mg^{2+}$ ions/100 nucleotides) and we estimate that for *E. coli* S-rRNA approx. seven nucleotides/100 residues are involved in the conformational change. If $n = 1$, i.e. if there is 1$Mg^{2+}$ ion bound/(sensitive) nucleotide, then a similar proportion of nucleotides are affected by $Mg^{2+}$ in both tRNA and rRNA. Römer & Hach (1975) concluded that tRNA tertiary structure corresponding to early 'melting' is stabilized by strongly bound $Mg^{2+}$, there being $5 \pm 1$ non-interacting strong sites per molecule. The sets of data for tRNA and rRNA are not inconsistent.

*Specificity of the interaction of cations with rRNA*

Repulsions between diesterified phosphate residues oppose bihelix formation, and, because cations modify the electrostatic field, the conformation of rRNA depends on electrolyte concentration. The influence of cations is not simply related to ionic strength, because bivalent cations are more than fifty times as efficient as univalent cations such as $K^+$ and $Na^+$ in inducing bihelical secondary structure in rRNA (Cox & Littauer, 1962; see also Table 1). Both $Na^+$ and $K^+$ affect the spectrum of *E. coli* rRNA to comparable extents (Cox & Littauer, 1962); and we have found that $Sr^{2+}$ is equivalent to $Mg^{2+}$ in its effect on the spectrum of L-rRNA in reconstitution buffer. Thus the combination of $Na^+$ and $Sr^{2+}$ would be expected to have much the same effects on the spectrum of rRNA as $K^+$ and $Mg^{2+}$. In 0.35M-KCl, both $Mg^{2+}$ and $Sr^{2+}$ affect the rRNA spectrum, and the results allow the possibility that $Mg^{2+}$ and $Sr^{2+}$, for example, exert specific effects (cf. the term $f_N(Mg^{2+})$ in eqn. 15). There may be specific effects that are not revealed by our spectrophotometric approach. The conformation of the active subribosomal particle may be more sensitive to specific cations than isolated rRNA, but we have not studied this point. However, experiments of the type summarized in Fig. 1, with different combinations of univalent and bivalent cations, might provide insight into specific ion effects.

*Disassembly of ribosomes by salt-shock treatment in the presence of sufficient $Mg^{2+}$ to maintain native rRNA conformation*

The search for well-defined core particles and split-protein fractions from eukaryotic ribosomes has not proved fruitful, although a wide range of conditions have been used (for a review, see Wool & Stöffler, 1974). The delineation of the conditions of $Mg^{2+}/K^+$ needed to maintain 'native' rRNA conformation has allowed us to examine the effect of increasing salt concentration when sufficient $Mg^{2+}$ was present to maintain the native conformation of the rRNA moiety of ribosomes. Thus one parameter was controlled and so provided a more rational basis for seeking useful core particles. The condition that the 'native' rRNA conformation is probably maintained only at suitable concentrations of $Mg^{2+}$ was expected to increase the chances of reconstructing active subparticles from core-particle and split-protein fractions. The expectation was justified by the isolation from rabbit L-subparticles of a core-particle and a split-protein fraction after treatment with 2.5M-$NH_4Cl$/62.5mM-$MgCl_2$ [see the next-but-one paper following (Cox & Greenwell, 1976)].

*Conclusion*

The idea that $Mg^{2+}$ affects mainly the rRNA moiety of ribosomes is not new. Our contribution is a quantitative analysis of the effect that $Mg^{2+}$ has on rRNA conformation and the recognition that the conformational change induced leads to an increased number of A·U base-pairs, resulting in a more compact conformation. The amount of $Mg^{2+}$ needed to effect the conformational change in rRNA, the $Mg^{2+}$ requirement for ribosome reassembly, and the concentration of $Mg^{2+}$ needed to stabilize ribosome function are all similar and so allow the possibility that $Mg^{2+}$ is needed to stabilize 'native' rRNA conformation; of course, $Mg^{2+}$ could fulfil other roles as well.

However, we need direct evidence that $Mg^{2+}$ induces the same conformational change in rRNA and in ribosomes. Such evidence was obtained through the study of the inactivation of the peptidyltransferase centre of rabbit L-subparticle on exposure to low concentrations of $Mg^{2+}$ (2mM-$MgCl_2$/0.5M-$NH_4Cl$). In this case restoring the concentration of $Mg^{2+}$ to 20mM brought about the same change in the u.v. spectrum as that found when $Mg^{2+}$ was added to L-rRNA [see the following paper (Cox *et al.*, 1976*b*)]. It appeared that the loss and regain of peptidyltransferase activity was concomitant with a conformational change in the rRNA moiety. The present paper provides further support for the view that rRNA conformation is important to ribosome function.

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