The size and conformation of *Artemia* (brine-shrimp) ribosomal RNA free in solution

Kristin DONCEEL, Paul NIEUWENHUYSEN and Julius CLAUWAERT
Department of Cell Biology, Universitaire Instelling Antwerpen, B-2610 Wilrijk, Belgium

(Received 5 March 1982/Accepted 4 June 1982)

The RNA was isolated from the large ribosomal subunits of the brine shrimp *Artemia*, and its conformation free in solution was studied by determining its sedimentation and diffusion coefficients. A comparison was made of the hydrodynamic radius of the ribosomal subunit and its isolated RNA in various buffers. The conformation of the rRNA free in solution is more extended than when it is incorporated in the ribosome. This is not only the case when the rRNA solution lacks bivalent and polyvalent cations, but even in the presence of Mg^{2+} and spermidine, which cause a tightening of RNA. Thus the ribosomal proteins should induce a further tightening of the rRNA during the assembly of the ribosome. In the discussion, the reported data on *Escherichia coli* rRNA species are presented in such a way that large discrepancies between various studies are revealed, and that they can be compared with the data reported here on the larger rRNA of an eukaryote.

The conformation of RNA free in solution must be known in order to understand the assembly of the RNA into biological structures such as viruses, ribosomes, messenger ribonucleoproteins or other RNA–protein complexes. The rRNA molecules of the prokaryote *Escherichia coli* have been mainly studied. The degree of unfolding of the RNA depends on the ionic concentrations of the solution; in particular, Mg^{2+} induces a tightening of the RNA (see, e.g., Cammack *et al.*, 1970; Schulte *et al.*, 1974; Allen & Wong, 1978; Tam *et al.*, 1981a). However, data reported by various groups vary greatly, even when the same RNA was studied in identical conditions (Allen & Wong, 1978; Bogdanov *et al.*, 1978; Tam *et al.*, 1981a). Thus opposing answers have been given to the interrelated questions: to what extent is the free RNA unfolded? how large are the conformational changes of the RNA during its assembly with ribosomal proteins into the ribosomal subunits?

In view of this controversial situation, we report here mainly a comparison of the size in solution of the large ribosomal subunit and of its free RNA, isolated from an eukaryote, the brine shrimp *Artemia*. This organism is widely used as a model system to solve biological problems. In particular, many studies have previously focused on protein synthesis, ribosomes and rRNA species of *Artemia* (e.g. Nieuwenhuysen & Clauwaert, 1978, 1981; Persoone *et al.*, 1980; Cruces *et al.*, 1982).

Our results favour the view that Mg^{2+} ions induce

a considerable tightening of the free RNA to a conformation that is still more extended than that found in the ribosome. Also we show discrepancies between the various reported data on *E. coli* rRNA species.

Parts of this study have been presented at the 7th International Biophysics Congress–3rd Pan-American Biochemistry Congress, Mexico, August 1981 (Donceel *et al.*, 1981), and at the 7th EMBO Annual Symposium, ‘Ribosome Structure and Function’, Heidelberg, September 1981.

Experimental

Preparation of rRNA

Ribosomal subunits were isolated from the cryptobiotic embryos of *Artemia* from San Francisco Bay as described by Nieuwenhuysen & Clauwaert (1981). They were concentrated by centrifugation and their RNA was extracted by using a method based on that of Slegers & Kondo (1977). A pellet of ribosomal subunits was dissolved in 10 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/KOH buffer (pH 7.5) containing 50 mM-KCl and 1 mM-EDTA. A 20% (w/v) solution of sodium dodecyl sulphate was added to give a final concentration of 2%. After 15 min at room temperature, the samples were put in an ice bath, and 1 vol. of redistilled phenol/chloroform (1:1, v/v) was added. The samples were centrifuged at 5000 *g* for 30 min for phase separation. The aqueous phase was treated again twice with the phenol/chloroform mixture.

RNA was recovered by precipitation overnight with 0.1 vol. of 20% (w/v) potassium acetate and 2 vol. of ethanol at -20°C . The RNA was dissolved in Hepes/KCl/EDTA buffer, and purified by rate-zonal centrifugation in a linear gradient of 10–30% (w/v) sucrose in the same buffer, at 67 000 *g* for 16 h in a Beckman SW40 rotor at 4°C . The fractions, containing rRNA, were dialysed overnight at 4°C against buffers specified below.

Alternatively, rRNA was extracted from the subunit by the sodium dodecyl sulphate procedure (Noll & Stutz, 1968), which allows the isolation of RNA without using EDTA. A pellet of ribosome subunits was dissolved in 10 mM-Hepes/KOH (pH 7.5)/50 mM-KCl/1 mM-EDTA, or in 20 mM-Tris/HCl (pH 7.5)/5 mM-MgCl₂ containing 50 mM-KCl or 50 mM-NH₄Cl. Then 20% sodium dodecyl sulphate solution was added to give a final concentration of 2%. The mixture was kept at 37°C for 2 min. The rRNA was further purified by rate zonal centrifugation as described above, in the buffer in which the ribosome subunits were dissolved. The rRNA fractions obtained were dialysed against buffers specified below.

Spectrophotometry

Absorbance spectra of the RNA solutions were measured with a Zeiss PMQ 3 spectrophotometer.

Analytical boundary sedimentation

The sedimentation coefficient and the degree of aggregation of the RNA in various buffers was measured by analytical boundary sedimentation. Runs were performed in a MSE analytical ultracentrifuge with a five-place rotor. Double-sector cells were used, with u.v.-absorption optics coupled to a photoelectric scanner. RNA concentrations were smaller than 40 $\mu\text{g}/\text{ml}$, so that concentration effects were negligible. The temperature was kept at $4.0 \pm 0.1^{\circ}\text{C}$ to avoid dissociation of the rRNA of the large subunit owing to the hidden break (Ishikawa, 1977). Temperature and solvent differences were taken into account (Owens *et al.*, 1980) to reduce the apparent sedimentation coefficient to $s_{20,w}^0$.

Photon correlation spectroscopy of scattered laser light

The translational diffusion coefficient, *D*, of the rRNA in Brownian motion was determined by analysing the intensity fluctuations of their laser light scattering, by using single-clipped photon-count autocorrelation spectroscopy (see, e.g., Koppel, 1974; Nieuwenhuysen, 1978; Nieuwenhuysen & Clauwaert, 1978). The measured correlation function decays exponentially with a decay time proportional to *D*:

$$G(iT) = A + B \exp(-2DK^2iT)$$

where *i* is the channel number, *T* the chosen sample time, and *K* the known modulus of the scattering vector; *A* and *B* are constants.

Calculation of hydrodynamic properties

The frictional coefficient ratio $f/f_{\min.}$ (e.g. Tanford, 1961) was calculated by using the standard sedimentation coefficient, $s_{20,w}^0$, in the equation

$$f/f_{\min.}(s) = \frac{(4/3)^{\dagger} (1 - \bar{v}\rho) M^{\dagger}}{6\eta(\pi N)^{\dagger} (\bar{v})^{\dagger} s_{20,w}^0}$$

or by using the standard diffusion coefficient, $D_{20,w}^0$, in the equation

$$f/f_{\min.}(D) = \frac{(4N/3)kT}{6\eta(\pi)^{\dagger}(\bar{v}M)^{\dagger} D_{20,w}^0}$$

where *M* is the molecular weight and $\bar{v}$ the partial specific volume of the macromolecule; η is the viscosity and ρ the density of water at a temperature, *T*, of 293 K; *k* is the Boltzmann constant, and *N* is Avogadro's number.

The hydrodynamic radius R_h was calculated by using the sedimentation coefficient in the equation

$$R_h(s) = \frac{M(1 - \bar{v}\rho)}{6\pi N \eta s_{20,w}^0}$$

or by using the diffusion coefficient in the equation

$$R_h(D) = \frac{kT}{6\pi\eta D_{20,w}^0}$$

where the symbols are the same as those defined above.

Results

The A_{260}/A_{280} of the rRNA solutions agrees with the expected value, obtained by extrapolation from data on ribonucleoproteins (Fig. 1); also, similar values have been reported for RNA of bacteriophages (reviewed by Fiers, 1979). This is satisfactory with regard to the purity of the rRNA preparations.

We have determined the hydrodynamic properties of the RNA in various concentrations of MgCl₂, mainly in 20 mM-Tris/HCl buffer (pH 7.5) containing 360 mM-KCl or 400 mM-NH₄Cl. This buffer and concentrations of univalent cations were chosen because (i) they are used for the reconstitution *in vitro* of *E. coli* small and large ribosomal subunits respectively, and (ii) these conditions were used in the most recent physicochemical studies of the rRNA of *E. coli* (see the Discussion section). However, Mg²⁺ induces the aggregation of *E. coli* rRNA (Cammack *et al.*, 1970; Schulte *et al.*, 1974; Pilz *et al.*, 1981; Tam *et al.*, 1981a), and also of *Artemia* rRNA, which hampers the measurements. Even with freshly prepared solutions of *Artemia* rRNA,

Fig. 1. Measured values for the A_{260} and A_{280} of RNA species and RNA-protein complexes

●, Ribosomal RNA isolated from *Artemia* (the present study); □, ribosomal particles of *Artemia* (Nieuwenhuysen & Clauwaert, 1981); ■, bacteriophage R17 (reviewed by Fiers, 1979). The linear relationship between the data plotted in this way is satisfactory, as outlined in Nieuwenhuysen & Clauwaert (1981).

at 4°C, we observed that the concentration of Mg^{2+} was increased from 0 to 20 mM, the aggregation of the large RNA of the large subunits increased from 0 to 15%, and that of the RNA on the small subunits from 0 to 100%. So we first studied the RNA of the large subunits.

In a buffer containing a low concentration of univalent cations and lacking Mg^{2+} (as used in the early experiments on RNA), the RNA has a sedimentation coefficient in the range of the values traditionally assigned to the large RNA of the large subunits of cytoplasmic eukaryotic ribosomes (Fig. 2a). Increasing the Mg^{2+} concentration leads to an increase in $s_{20,w}^0$ up to 34.5 S (Fig. 2a); this indicates a tightening of the RNA. No further increase in $s_{20,w}^0$ was measured when 2 mM-spermidine was added to the buffer containing 20 mM- $MgCl_2$; this contrasts with the report that 2 mM-spermidine caused a further tightening of the RNA of the large ribosomal subunit of *E. coli*, which could not be achieved by adding more Mg^{2+} to the buffer (Vasiliev & Zalite, 1980). The degree of unfolding depends less on univalent than on bivalent ions: (i) decreasing the concentration of KCl from 360 to 100 mM at a constant 20 mM- $MgCl_2$ caused a decrease in $s_{20,w}^0$ from only 34.5 S to 33.5 S; (ii) substituting NH_4Cl for KCl caused only a small or no change in $s_{20,w}^0$ (Fig. 2a). The isolation method with phenol and EDTA yielded RNA that was somewhat more dissociated (owing to the hidden break) than did the more direct extraction method using only sodium dodecyl sulphate without EDTA; however, no differences were finally measured in their sedimentation coefficient.

To allow a more quantitative interpretation of the measured values for $s_{20,w}^0$, these were used to calculate the hydrodynamic properties of the RNA,

Fig. 2. Hydrodynamic properties of the large RNA of the large ribosomal subunits of *Artemia*, in 20 mM-Tris/HCl buffer (pH 7.5), containing concentrations of $MgCl_2$ as indicated, and 360 mM-KCl (●), 400 mM- NH_4Cl (□) or 400 mM- NH_4Cl and 0.2 mM-EDTA (■), or in 10 mM-Hepes/KOH buffer (pH 7.5), containing 50 mM-KCl and 1 mM-EDTA (▲)

(a) $s_{20,w}^0$ is the sedimentation coefficient of the RNA at infinite dilution, measured at 4°C and reduced to standard conditions. (b) f/f_{min} is the frictional coefficient ratio of the RNA, calculated from $s_{20,w}^0$, the molecular weight and the partial specific volume. The decrease in f/f_{min} reflects the tightening of the rRNA. (c) R_h is the hydrodynamic radius of the RNA, calculated from $s_{20,w}^0$, the molecular weight and the partial specific volume. R_h reflects the size in solution. For comparison, the horizontal lines indicate the measured value (and estimated accuracy) for the hydrodynamic radius of the large ribosomal subunit of *Artemia* (Nieuwenhuysen & Clauwaert, 1981). The error bars indicate the uncertainty in the hydrodynamic properties calculated only from the sedimentation coefficient; however, the final uncertainty is smaller, in view of the agreement with the value for R_h determined directly by photon correlation spectroscopy (see the Results section).

by using the equations in the Experimental section. In order to apply these equations, the molecular weight and the partial specific volume of the RNA

must be known. To the molecular weight we can assign a value of 1.23×10^6 , calculated by combining the total molecular weight of the large ribosomal subunit with its ratio of RNA to protein content, which have been determined in our laboratory (Nieuwenhuysen & Clauwaert, 1981), and by taking into account the contribution of the 5S RNA. This value agrees with those determined by gel electrophoresis in denaturing conditions (Camarano *et al.*, 1975; Cruces *et al.*, 1982), where the migration rate of *Artemia* rRNA is compared with that of *E. coli* rRNA species, provided that the values for the molecular weight of the *E. coli* rRNA are considered which can now be calculated from the reported nucleotide sequences (Brosius *et al.*, 1978, 1980).

The wide range of the reported values for the partial specific volume, $\bar{v}$, causes a larger uncertainty in the calculated hydrodynamic properties of the RNA. Such a large variation in $\bar{v}$ values is not surprising in view of the differences in the origin, degree of purity and the kind of counterions of the studied RNA, and of the experimental difficulties and variations in the determination of $\bar{v}$. In the calculations we have allowed $\bar{v}$ to vary in the range of values from 0.53 to 0.58 cm³/g; this spread covers the reported values for K⁺ salts of rRNA (see the compilation by Nieuwenhuysen & Clauwaert, 1981) and includes the most recent values for *E. coli* rRNA species (Tam *et al.*, 1981a,b).

The calculated values for the frictional coefficient ratio $f/f_{\min.}$ and for the hydrodynamic radius R_h of the RNA are plotted in Figs. 2(b) and 2(c) respectively. The error bars indicate the uncertainty in these values calculated from $s_{20,w}^0$, M and $\bar{v}$. However, the actual uncertainty is smaller, as explained hereafter. Photon correlation spectroscopy of scattered laser light could be used to measure directly the diffusion coefficient D and thus also R_h of the monodisperse rRNA in 10 mM-Hepes/KOH (pH 7.5)/50 mM-KCl/1 mM-EDTA, since this buffer causes no aggregation, as checked by analytical boundary centrifugation. The concentration of the rRNA sample investigated was as low as 0.4 mg/ml, so that the concentration-dependence of D was negligible (e.g. Hill *et al.*, 1977). The spectra obtained at scattering angles between 45° and 135° decayed monoexponentially and yielded identical values for R_h of 20.8 ± 0.4 nm. This agrees well with the value of 20.7 ± 1.5 nm, which is calculated from $s_{20,w}^0$, M and $\bar{v}$.

Like the values for $s_{20,w}^0$, the decreasing $f/f_{\min.}$ and R_h reflect the tightening of the RNA with increasing concentration of Mg²⁺. Furthermore, unlike $s_{20,w}^0$, the quantity $f/f_{\min.}$ allows a comparison, in the Discussion section, with the degree of unfolding of other rRNA species, irrespective of their molecular weight. Also unlike $s_{20,w}^0$, the hydrodynamic radius R_h reflects exclusively the dimen-

sions in solution of the RNA in various Mg²⁺ concentrations. This allows a direct comparison with R_h of the large ribosomal subunits, which has been determined in our laboratory by photon correlation spectroscopy of laser light scattered by the subunits in solution (Nieuwenhuysen & Clauwaert, 1981), and which is indicated in Fig. 2(c) by the horizontal line. It turns out that R_h of the large subunit is somewhat smaller than R_h of their rRNA free in solution at 4°C. Now the following facts should also be considered.

(1) As much as 48% of the large ribosomal subunit of *Artemia* is made up of proteins (Nieuwenhuysen & Clauwaert, 1981).

(2) The central regions of ribosomal particles are RNA-rich, whereas their exteriors are protein-rich (e.g. Serduyk & Grenader, 1975; Crichton *et al.*, 1977).

(3) Ribosomes are assembled at a temperature higher than our measurement temperature, 4°C; whereas the size of the ribosomal subunits is not sensitive to temperature (e.g. Allen & Wong, 1979), it has been reported that rRNA is unfolded with increasing temperature (Schulte *et al.*, 1974; Tam *et al.*, 1981a).

(4) R_h of a macromolecule represents only the radius of a hydrodynamically equivalent sphere; in case of asymmetry of the macromolecule, here the rRNA free in solution, its maximal dimension is greater than R_h (see, e.g., Tanford, 1961).

So our data and these facts together indicate that the rRNA free in solution assumes a more loose conformation than when it is incorporated in the ribosome; this picture applies not only to rRNA in buffers lacking Mg²⁺, but also in buffers that contain higher concentrations of univalent cations, Mg²⁺ and even spermidine, which neutralize the repelling negative charges on the polyribonucleotide backbone.

Discussion

First we consider the values measured for the sedimentation and/or diffusion coefficient of the ribosomal subunits and RNA species of *E. coli* (Table 1). From these data their hydrodynamic properties can be calculated, by using the equations in the Experimental section, after assigning values to their molecular weight and their partial specific volume (Table 2). The resulting calculated values for the frictional coefficient ratio $f/f_{\min.}$ and the hydrodynamic radius R_h of the ribosomal subunits and their RNA are also listed in Table 1. The data reported for the ribosomal subunits show relatively small variations. These can at least partly be attributed to differences in preparation procedure, buffer composition and temperature of measurement. So from this compilation of data, a rather narrow range is obtained of reasonable values for the hydro-

Table 1. *Hydrodynamic properties of ribosomal subunits and their RNA molecules of E. coli and Artemia*

Buffers: a, 0.02 M-Tris/HCl (pH 7.5), 0.02 M-MgCl₂, 0.36 M-KCl; b, 0.02 M-Tris/HCl (pH 7.5), 0.02 M-MgCl₂, 0.05 M-KCl; c, 0.03 M-ammonium acetate, 0.006 M-magnesium acetate, 1 M-ethanol (pH 7.5); d, 0.01 M-Tris/HCl (pH 7.4), 0.1 M-KCl, 0.0015 M-MgCl₂; e, 0.05 M-ammonium acetate, 0.01 M-magnesium acetate, 1 M-ethanol (pH 7.5); f, 0.01 M-Tris/HCl (pH 7.5), 0.01 M-NH₄Cl, 0.001 M-magnesium acetate; g, c + 0.002 M-spermidine; h, 0.02 M-Tris/HCl (pH 7.6), 0.004 M-magnesium acetate, 0.4 M-NH₄Cl, 0.0002 M-EDTA, 0.002 M-β-mercaptoethanol; i, 0.02 M-Hepes/KOH (pH 7.5), 0.25 M-KCl, 0.011 M-magnesium acetate; j, 0.01 M-Tris/HCl (pH 7.5), 0.05 M-NH₄Cl, 0.25 mM-MgCl₂; k, 0.01 M-Hepes/KOH (pH 7.5), 1 mM-EDTA, 0.05 M-KCl.

Reference	Temp. Buffer (°C)	$s_{20,w}^0$ (S)	$10^7 \times D_{20,w}^0$ (cm ² /s)	$f/f_{min.}$ (s)	$f/f_{min.}$ (D)	R_h (nm)	$\frac{R_h \text{ (RNA)}}{R_h \text{ (subunit)}}$
<i>Escherichia coli</i>							
Small ribosomal subunit							
Koppel (1974)	j 5	—	2.18 ± 0.03	—	1.64–1.72	9.6–10.0	—
Bogdanov <i>et al.</i> (1978)	a 20	—	2.02 ± 0.02	—	1.77–1.85	10.4–10.7	—
Allen & Wong (1979)	a 25	32.1 ± 0.4	—	1.52–1.71	—	9.0–9.9	—
	a 37	32.6 ± 0.4	—	1.50–1.68	—	8.8–9.7	—
b 25	b 25	35.6 ± 0.2	—	1.38–1.53	—	8.1–8.9	—
	a 4	31.8 ± 0.4	1.97 ± 0.03	1.53–1.73	1.81–1.90	9.0–11.0	—
Tam <i>et al.</i> (1981a)	a 4	31.8 ± 0.4	1.97 ± 0.03	1.53–1.73	1.81–1.90	9.0–11.0	—
RNA of the small ribosomal subunit							
Allen & Wong (1978)	a 37	21.1	—	1.85–2.16	—	9.1–10.4	0.82–1.28
Bogdanov <i>et al.</i> (1978)	a 20	—	1.96 ± 0.1	—	2.11–2.41	10.4–11.5	0.95–1.42
Vasiliev <i>et al.</i> (1978)	c 20	21.5	—	1.81–2.12	—	8.9–10.3	0.80–1.27
Tam <i>et al.</i> (1981a)	a 4	21.0 ± 0.8	1.72 ± 0.04	1.79–2.23	2.46–2.67	8.8–12.8	0.80–1.58
	a 37	18.4 ± 0.1	1.39 ± 0.1	2.11–2.46	2.91–3.47	10.4–16.6	0.94–2.05
Large ribosomal subunit							
Koppel (1974)	j 5	—	1.90 ± 0.03	—	1.57–1.65	11.0–11.5	—
Allen & Wong (1979)	a 25	49.9 ± 0.3	—	1.40–1.56	—	10.0–10.8	—
	a 37	49.4 ± 0.1	—	1.42–1.57	—	10.1–10.9	—
	b 25	49.7 ± 0.2	—	1.41–1.56	—	10.0–10.8	—
Tam & Hill (1980)	d 4	50.2 ± 0.5	1.90 ± 0.04	1.39–1.56	1.56–1.66	9.8–11.5	—
P. Nieuwenhuysen & J. Clauwaert (unpublished work)	f 20	49	1.90	1.44–1.58	1.57–1.65	10.1–11.5	—
Large RNA of the large ribosomal subunit							
Vasiliev & Zalite (1980)	e 20	31.0 ± 0.5	—	1.90–2.27	—	11.6–13.4	1.01–1.36
	g 20	34.0 ± 0.5	—	1.74–2.07	—	10.6–12.2	0.92–1.25
Tam <i>et al.</i> (1981b)	d 20	25.7 ± 0.4	1.33 ± 0.02	2.30–2.73	2.59–2.76	14.0–16.3	1.21–1.66
	h 20	26.3 ± 0.3	1.34 ± 0.04	2.25–2.66	2.54–2.66	13.7–16.4	1.19–1.67
<i>Artemia</i>							
Large ribosomal subunit							
Nieuwenhuysen & Clauwaert (1981)	i 20	59 ± 1	1.61 ± 0.03	—	1.51–1.69	13.0–13.5	—
Large RNA of the large ribosomal subunit							
This work	a 4	34.5 ± 0.4	—	1.98–2.35	—	13.0–14.9	0.96–1.15
	k 4	24.5 ± 0.3	1.03 ± 0.02	2.75–3.40	3.02–3.28	19.2–22.2	1.28–1.71

dynamic radius R_h of the small and large subunits. In contrast, however, relatively large differences are seen between the reported values for the rRNA molecules. This is considered further in more detail. Vasiliev and co-workers (Vasiliev *et al.*, 1978; Vasiliev & Zalite, 1980) have obtained rRNA molecules free in solution, in a specific compact conformation which resembles the size and shape of the ribosomal subunits as observed by electron microscopy; however, the RNA solutions contained 1 M-ethanol, which may cause conformational changes unrelated

to the situation *in vivo*. Three other groups have reported the sedimentation and/or diffusion coefficient of the rRNA of the small subunits in an identical buffer (Allen & Wong, 1978; Bogdanov *et al.*, 1978; Tam *et al.*, 1981a). However, their data vary widely and lead to a value for the hydrodynamic radius of the rRNA which is similar to (Allen & Wong, 1978), somewhat higher (Bogdanov *et al.*, 1978) or even much higher than the hydrodynamic radius of the whole subunit (Tam *et al.*, 1981a). So these conflicting results have lead to

Table 2. Values assigned to the molecular weight, M , and the partial specific volume, $\bar{v}$, of ribosomal subunits and RNA molecules of *E. coli*, in the calculations of their hydrodynamic properties, listed in Table 1

	$10^{-6} \times M$	$\bar{v}$ (cm ³ /g)
Small subunit	0.85 ^a	0.58–0.61 ^c
Large subunit	1.45 ^a	
RNA of the small subunit	0.52 ^b	
Large RNA of the large subunit	0.99 ^b	

^a Calculated from the primary structure of their RNAs (Brosius *et al.*, 1978, 1980) and proteins (H. G. Wittmann, unpublished work, communicated at the 7th Annual EMBO Symposium 'Ribosome Structure and Function', Heidelberg, September 1981).

^b Calculated from their nucleotide sequence (Brosius *et al.*, 1978, 1980).

^c This range covers the reported values, including the most recent ones (Tam & Hill, 1980; Tam *et al.*, 1981a; Tardieu *et al.*, 1981).

^d This range covers the reported values for the K⁺ salt of rRNA (compiled by Nieuwenhuysen & Clauwaert, 1981), and includes the most recent values for *E. coli* rRNA species (Tam *et al.*, 1981a,b).

opposing answers to the recurring question of whether the conformation of the free RNA is as compact as that found in the completed subunit, or whether the ribosomal proteins cause a tightening of the unfolded free rRNA, when they bind to it to assemble the ribosomal subunit. The most recent of those results indicate that in reconstitution conditions the conformation of the free rRNA is considerably more extended than in the completed small subunit (Tam *et al.*, 1981a). The same conclusion was reached by the same group for the larger rRNA of the large ribosomal subunit of *E. coli* (Tam *et al.*, 1981b) (Table 1).

Our results for the large RNA of the large subunit of the eukaryote *Artemia* lead to $f/f_{\min}$ values that lie in the range recalculated from the data reported for *E. coli* rRNA species in the same conditions (Table 1). This indicates similar degrees of unfolding of the different rRNA species. So our conclusion seems valid for various rRNA species. The results reported here favour the view that Mg²⁺ ions induce a marked tightening of the free rRNA, but that the conformation assumed by the rRNA free in solution is still more extended than that found in the completed ribosome, so that the ribosomal proteins, which bind to the rRNA during the assembly of the ribosome, cause a further compaction of the rRNA.

A very recent unpublished neutron-scattering study of the rRNA and rRNA-protein reconstitution intermediates of the small ribosomal subunit of *E. coli* supports our conclusion (Agalarov, S. C., Serduyk, I. N., May, R., Sedelnikova, S. E. & Spirin, A. S., poster contribution at the 7th

Annual EMBO Symposium 'Ribosome Structure and Function', Heidelberg, September 1981).

Skilful technical assistance was provided by Mr. T. Aerts. K. D. is a predoctoral fellow of the I.W.O.N.L./I.R.S.I.A. During this work P. N. was a Senior Research Assistant for the National Fund for Scientific Research (Belgium). This work was partially supported by grants from the F.K.F.O. (Belgium).

References

- Allen, S. H. & Wong, K. P. (1978) *J. Biol. Chem.* **253**, 8759–8766
- Allen, S. H. & Wong, K. P. (1979) *Arch. Biochem. Biophys.* **195**, 112–120
- Bogdanov, A. A., Zimmerman, R. A., Wang, C.-C. & Ford, N. C. (1978) *Science* **202**, 999–1001
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4801–4805
- Brosius, J., Dull, T. J. & Noller, H. F. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 201–204
- Cammack, K. A., Miller, D. S. & Grinstead, K. H. (1970) *Biochem. J.* **117**, 745–755
- Cammarano, P., Pons, S. & Londei, P. (1975) *Acta Biol. Med. Germ.* **34**, 1123–1135
- Crichton, R. R., Engelman, D. M., Haas, J., Koch, M. H. J., Moore, P. B., Parfait, R. & Stuhmann, H. B. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5547–5550
- Cruces, J., Sebastian, J. & Renart, J. (1982) *FEBS Lett.* **137**, 108–110
- Donceel, K., Nieuwenhuysen, P. & Clauwaert, J. (1981) *Abstr. Int. Biophys. Congr. 7th–Pan-Am. Biochem. Congr. 3rd, Mexico City*, p. 268
- Fiers, W. (1979) *Compr. Virol.* **13**, 69–204
- Hill, W. E., Bakke, K. R. & Blair, D. P. (1977) *Nucleic Acids Res.* **5**, 473–476
- Ishikawa, H. (1977) *Comp. Biochem. Physiol. B.* **56**, 229–234
- Koppel, D. E. (1974) *Biochemistry* **13**, 2712–2719
- Nieuwenhuysen, P. (1978) *Macromolecules* **11**, 832–834
- Nieuwenhuysen, P. & Clauwaert, J. (1978) *Biochemistry* **17**, 4260–4265
- Nieuwenhuysen, P. & Clauwaert, J. (1981) *J. Biol. Chem.* **256**, 9626–9633
- Noll, H. & Stutz, E. (1968) *Methods Enzymol.* **12**, 129–155
- Owens, D. P., Fox, J. W. & Wong, K. P. (1980) *Biochem. Biophys. Res. Commun.* **96**, 685–691
- Persoone, G., Sorgeloos, P., Roels, O. & Jaspers, E. (eds.) (1980) *The Brine Shrimp Artemia*, vols. 1–3, Universa Press, Wetteren
- Pilz, I., Wilhelm, P., Folkhard, W. & van de Haar, F. (1981) *Abstr. Int. Biophys. Congr. 7th–Pan-Am. Biochem. Congr. 3rd, Mexico City*, pp. 220–221
- Schulte, C., Morrison, C. A. & Garrett, R. A. (1974) *Biochemistry* **13**, 1032–1037
- Serduyk, I. N. & Grenader, A. K. (1975) *FEBS Lett.* **59**, 133–136
- Slegers, H. & Kondo, M. (1977) *Nucleic Acids Res.* **4**, 625–639
- Tam, M. F. & Hill, W. E. (1980) *FEBS Lett.* **120**, 230–232

- Tam, M. F., Dodd, J. A. & Hill, W. E. (1981a) *J. Biol. Chem.* **256**, 6430–6434
- Tam, M. F., Dodd, J. A. & Hill, W. E. (1981b) *FEBS Lett.* **130**, 217–220
- Tanford, C. (1961) *Physical Chemistry of Macromolecules*, pp. 317–412, John Wiley and Sons, New York
- Tardieu, A., Vachette, P., Gulik, A. & Le Maire, M. (1981) *Biochemistry* **20**, 4399–4406
- Vasiliev, V. D. & Zalite, O. M. (1980) *FEBS Lett.* **121**, 101–104
- Vasiliev, V. D., Selivanova, O. M. & Koteliensky, V. E. (1978) *FEBS Lett.* **95**, 273–275