α-MDM. On the basis of the dependence of the kinetics of $Ca^{2+}$ replacement on saccharide concentration, we concluded that α-MDM must also bind tightly to apo-Con A in the locked conformation, in fact, about 7% as well as to native Con A but ~ $10^3$ times more tightly than to apo-Con A in the unlocked conformation. In addition, we demonstrated the preferential binding of α-MDM to locked apo-Con A directly: α-MDM added to P was shown to drive the equilibrium P ⇄ PL + Sa ⇄ SaPL completely to the right. That SaPL was indeed formed was demonstrated by the relaxation behavior of these solutions upon the addition of appropriate amounts of $Mn^{2+}$ and $Ca^{2+}$ ions, the behavior of which is unique and predictable on the basis of our earlier work (Brown et al., 1977). Finally, we have extended the multiple equilibria schemes, first introduced by Brown et al. (1977) to explain the interaction of $Mn^{2+}$ and $Ca^{2+}$ ions with Con A in its two conformations, to include competitive interactions for identical metal-binding sites, and to include interactions with saccharides as well.

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References

Alter, G. M., & Magnuson, J. A. (1974) *Biochemistry 13,* 4038-4045.
Alter, G. M., Pandolfino, E. R., Christie, D. J., & Magnuson, J. A. (1977) *Biochemistry 16,* 4034-4038.
Bessler, W., Shafer, J. A., & Goldstein, I. J. (1974) *J. Biol. Chem. 249,* 2819-2822.
Bittiger, H., & Schnebli, H. P. (1976) *Concanavalin A as a Tool,* Wiley, New York, N.Y.
Brewer, C. F., Sternlicht, H., Marcus, D. M., & Grollman, A. P. (1973a) *Proc. Natl. Acad. Sci. U.S.A. 70,* 1007-1011.
Brewer, C. F., Sternlicht, H., Marcus, D. M., & Grollman, A. P. (1973b) *Biochemistry 12,* 4448-4457.
Brown, R. D., III, Brewer, C. F., & Koenig, S. H. (1977) *Biochemistry 16,* 3883-3896.
Grimaldi, J. J., & Sykes, B. D. (1975) *J. Biol. Chem. 250,* 1618-1624.
Hallenga, K., & Koenig, S. H. (1976) *Biochemistry 15,* 4255-4264.
Harrington, P. C., & Wilkins, R. G. (1978) *Biochemistry 17* (preceding paper in this issue).
Kalb, A. J., & Levitzki, A. (1968) *Biochem. J. 109,* 669-672.
Koenig, S. H., Brown, R. D., & Studebaker, J. (1971) *Cold Spring Harbor Symp. Quant. Biol. 36,* 551-559.
Koenig, S. H., Brown, R. D., & Brewer, C. F. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70,* 475-479.
Loontiens, G. F., Clegg, R. M., van Landschoot, A., & Jovin, T. M. (1977) *Eur. J. Biochem. 78,* 465-469.
Reeke, G. N., Jr., Becker, J. W., & Edelman, G. M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75,* 2286-2290.
Richardson, C. E., & Behnke, W. D. (1976) *J. Mol. Biol. 102,* 441-451.
Rubinstein, M., Baram, A., & Luz, Z. (1971) *Mol. Phys. 20,* 67-80.
Shoham, M., Kalb, A. J., & Pecht, I. (1973) *Biochemistry 12,* 1914-1917.
Villafranca, J. J., & Viola, R. E. (1974) *Arch. Biochem. Biophys. 160,* 465-468.
Yariv, J., Kalb, A. J., & Levitzki, A. (1968) *Biochim. Biophys. Acta 165,* 303-305.
Zimmerman, S. S., & Scheraga, H. A. (1976) *Macromolecules 9,* 408-416.

# Physical Properties of *Artemia salina* Ribosomes†

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ABSTRACT: Eukaryotic ribosomes were isolated from the cryptobiotic embryos and from the further-developed free-swimming nauplii of the brine shrimp *Artemia salina.* Analytical boundary sedimentation and photon correlation spectroscopy yielded, respectively, the standard sedimentation and diffusion coefficients at infinite dilution, $s^\circ_{20,w} = 81 \pm 1$ S and $D^\circ_{20,w} = (1.41 \pm 0.02) \times 10^{-7}$ cm²/s, for the unfixed and formaldehyde-fixed ribosomes from different developmental stages and for ribosomes attached to a messenger RNA fragment. Also, the density increment was determined, from which the partial specific volume was derived (0.63 ± 0.01 cm³/g). Combination of the different measured parameters gives accurate values for the molecular weight $(3.8 \pm 0.1) \times 10^6$ and for size and solvation parameters. These results are compared with their counterparts for the smaller ribosomes from the prokaryote *Escherichia coli.*

The ribosome is a subcellular particle containing RNA and proteins. Engaged in protein synthesis, as a part of a polysome, it is the site of translation of messenger RNA into polypeptide sequences. It is already possible to establish a coherent picture of the physical properties of the ribosomes isolated from the prokaryote *Escherichia coli,* but the information about the larger ribosomes from eukaryotes is still much more limited (Van Holde and Hill, 1974). The difficulties in physical-chemical studies of ribosomes are caused by contamination of the solutions by other subcellular particles, by a possible conformational difference between free single ribosomes and those attached to mRNA in polysomes (Vournakis and Rich, 1971), by the pressure-dependent equilibrium between the ribosomes

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and their large and small subunits, by aggregation and denaturation of ribosomal particles, etc. Even the comparison of results is made difficult by variations in the source of ribosomes, the isolation procedure, the solvents, the degree of purity, the methods of characterization, etc. As will be pointed out throughout this paper, we have excluded the ambiguity arising from the above-mentioned different forms of polydispersity in our study of eukaryotic ribosomes, isolated from cysts and nauplii of the brine shrimp *Artemia salina*. Generally, our motivation for the choice of this source of ribosomes is the great interest in *A. salina* for studies of protein synthesis and cell differentiation (Hentschel and Tata, 1976); more specifically, the undeveloped embryos of *A. salina* are rich in free single ribosomes, which are not bound to membranes, and they contain virtually no polysomes (Hultin and Morris, 1968); ribosomes and polysomes can, however, also be isolated from *A. salina* in a further stage of development, e.g., from the free-swimming nauplii. We present here accurate measurements of the sedimentation and diffusion coefficients and the density increment of the ribosomes, from which we derive their molecular weight, size, and solvation. These results can then be compared with their counterparts for *E. coli* ribosomes.

## Experimental Procedure

*Preparation of Ribosome Solutions.* The different kinds of ribosomes were prepared starting from cryptobiotic embryos of *A. salina* (Hultin and Morris, 1968; Hentschel and Tata, 1976) from the San Francisco Bay. The dry cysts contain a large supply of free ribosomes, very few membrane-associated ribosomal particles, and virtually no free polysomes (Hultin and Morris, 1968).

In all steps of the different preparations and during the measurements, the ribosome solutions contained 20 mM Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.5), 9 mM magnesium acetate, 70 mM KCl, and 1 mM dithiothreitol.

For the direct isolation of ribosomes from the undeveloped cryptobiotic embryos, we followed a procedure, developed in our laboratory, which yields very pure monodisperse ribosome solutions (Nieuwenhuysen and Slegers, 1978). In short, this procedure involves washing and grinding the cysts, preparation of the PMS[1] with differential pelletings, isopycnic centrifugation of the PMS in a sucrose density gradient, precipitation of the ribosomal fractions with PEG 6000, and zonal sucrose density gradient centrifugation of the resuspended ribosomes.

For the isolation of ribosomes from further-developed embryos, the cysts were first suspended and incubated at 30 °C in a medium containing artificial sea salt until the hatching of swimming nauplii. These nauplii were collected for the preparation of polysomes. The ribosomal particles were pelleted from the PMS in a Spinco Ti 60 fixed-angle rotor. Ribosomes and polysomes of different order were separated by sucrose density gradient centrifugation of the resuspended particles in a Spinco Ti 14 zonal rotor. The ribosomes from the fractions in the 80S region were precipitated with PEG, resuspended, and subjected to another sucrose density gradient centrifugation. This finally yielded what we will call further ribosomes from nauplii. To obtain only ribosomes attached to messenger RNA, polysomes containing four or more ribosomes were incubated at 25 °C for 10 min with a minimal concentration of insolubilized pancreatic ribonuclease. (This enzyme has a great affinity for the exposed single-stranded messenger RNA.) Subsequently, the fiber-bound RNase was removed by low-speed centrifugation. The optimal fiber concentration, i.e., that which is needed for nearly complete conversion of polysomes into ribosomes, was determined earlier in preliminary experiments by analyzing polysomes, incubated with different fiber concentrations, by sucrose density gradient sedimentation in a Spinco SW 27 or SW 41 rotor.

Ribosomes were fixed by dialysis of dilute solutions in the cold for 24 h against our standard buffer containing 5% freshly neutralized formaldehyde. The excess of formaldehyde was removed by further dialysis against buffer solutions (Nieuwenhuysen et al., 1978a).

The integrity and purity, and the absence of aggregation and dissociation of the ribosomes isolated from cryptobiotic embryos or from nauplii, were checked with UV spectroscopy, zonal sucrose gradient centrifugation, analytical CsCl isodensity equilibrium centrifugation, and electron microscopy as described elsewhere (Nieuwenhuysen and Slegers, 1978) and with analytical boundary sedimentation and photon correlation spectroscopy as described below in more detail.

Prior to the measurements, the solutions were dialyzed against our standard buffer. The absence of residual sucrose was checked by refractometry.

*Analytical Boundary Sedimentation.* The sedimentation coefficients were measured by boundary sedimentation using an MSE analytical ultracentrifuge with double-sector cells and absorption optics at 260 nm in combination with an automatic photoelectric scanning device. Rotor speeds ranged from 12 000 to 36 000 rpm. Temperatures were controlled within 0.1 °C between 15 and 25 °C. The use of a four- or six-hole rotor allowed simultaneous measurements on different samples. The concentration of ribosomes ranged from 40 to 80 μg/mL, so that no concentration effects had to be taken into account. Reduction of the sedimentation coefficient to standard conditions of 20 °C and water yielded $s^\circ_{20,w}$.

These measurements also allow us at the same time to check the absence of aggregation and dissociation, the purity, and the integrity of the ribosomes. So they were performed prior to photon correlation spectroscopy and measurements of the density increment.

*Photon Correlation Spectroscopy.* Single-clipped photon-count autocorrelation spectra (Foord et al., 1970) were obtained with a setup from Malvern Instruments (Worcs, England) in a thermostated room. In the preparative stage of the measurements, several steps were taken to avoid dust particles in the light-scattering volume. First the ribosomal solutions were centrifuged at speeds higher than 12 000 rpm in a fixed-angle rotor at 4 °C. Then, 2 mL was carefully pipetted out of the middle of the tube into a cylindrical scattering cell which had been cleaned with freshly distilled acetone. A specially constructed adapter allowed a subsequent centrifugation of the filled cell up to 6000 rpm in a Beckman JA 21 centrifuge. Afterwards, the cell was immersed in an index matching water bath, carefully avoiding any impact so that the eventually present dust particles remained at the bottom due to the final centrifugation. The temperature was maintained at 25.0 ± 0.1 °C with a Malvern temperature controller. A beam of light with wavelength $\lambda_0$ 4880 Å of 40 to 150 mW from an intensity-stabilized Coherent Radiation argon ion laser was focused in the cell. The scattered light was detected with an ITT FW130 photomultiplier, which is excellent for this application (Foord et al., 1969). The single-clipped autocorrelation function of the photon counts was built up in a Malvern 24-channel digital correlator (Foord et al., 1970). The sample times $T$ and clipping levels $k$ were chosen to yield optimal accuracy (Hughes et al., 1973). Autocorrelation spectra were

[1] Abbreviations used are: PMS, postmitochondrial supernatant; PEG, poly(ethylene glycol); BSA, bovine serum albumin.

taken at scattering angles $\theta$ between 30° and 145° and were recorded on paper tape for subsequent analysis with a PDP 11/45 computer.

Theoretical considerations have yielded the following relation for the normalized single-clipped photon count autocorrelation function $g_k^{(2)}(iT)$, with $i = 1, 2, \ldots, 24$ for our setup, of the light scattered by a dilute monodisperse solution of small Brownian particles with diffusion coefficient $D$ (Foord et al., 1970; Pusey et al., 1974; Koppel, 1974).

$$g_k^{(2)}(iT) - 1 = A \exp(-2D\mathbf{K}^2 iT)$$

In this expression, $A$ is an experimental constant depending on the mean number of counts per sample time, the decay time, $T$, and $\mathbf{K}$, and on the geometrical arrangement of the optical components (Nieuwenhuysen and Clauwaert, 1977); the modulus of the scattering vector $\mathbf{K}$ is given by

$$\mathbf{K} = (4\pi/\lambda_0)n \sin(\theta/2)$$

with $n$ the solution refractive index. In our data-analysis method, described elsewhere (Nieuwenhuysen and Clauwaert, 1978a; Nieuwenhuysen, 1978), we combined unweighted and iteratively weighted least-squares fitting procedures of the logarithm of $g_k^{(2)}(iT) - 1$ to straight lines and quadratic curves.

The reliability of our apparatus and the data analysis procedure were checked previously with experiments on monodisperse 91-nm polystyrene latex spheres (Nieuwenhuysen and Clauwaert, 1977, 1978a; Nieuwenhuysen, 1978) and MS2 virus (Nieuwenhuysen and Clauwaert, 1978b). The light scattered from benzene yielded the expected angular distribution of photon counts. Moreover, the diffusion coefficients of ribosomes were also measured with a modified Malvern setup (Jolly and Eisenberg, 1976). There, the light source was an intensity-stabilized Spectra Physics argon ion laser, emitting light with $\lambda_0$ 5145 Å. Large high-quality Sofica scattering cells were used, painstakingly cleaned with a flow of filtered, twice-distilled water, following the complete procedure of Dubin et al. (1971). The index matching bath was filled with toluene, which was kept constant to within 0.1 °C, close to 20 °C. Spectra were analyzed directly with a desk-top computer, mainly by weighted least-square fits of $\ln[g_k^{(2)}(iT) - 1]$ to quadratic curves.

Reduction of the diffusion coefficient to standard conditions of water at 20 °C yielded $D_{20,w}$ (Pusey et al., 1974; Koppel, 1974).

*Density and Concentration Measurements.* The density increment at zero concentration and constant chemical potential of all diffusible components $(\partial\rho/\partial c)_\mu^0$ (Eisenberg, 1976) was obtained from a density vs. concentration plot. The solutions were brought to thermodynamic equilibrium of diffusible components by exhaustive dialysis against buffer.

The densities were determined with an Anton Paar digital precision density meter DMA 02C (Kratky et al., 1973) in a thermostated room. The temperature was kept constant within 0.005 °C at 25.0 °C using a Tronac precision temperature controller Model 40 and two thermostates in series. Dry air and twice-distilled water, made dust free by filtration through a 0.1-$\mu$m Millipore filter and subsequent centrifugation, were used for the calibration of the instrument. The ribosomal solutions were made dust free by centrifugation.

The precision of the $(\partial\rho/\partial c)_\mu^0$ determination is limited here by the precision of the concentration measurements. We determined the concentration of the ribosomes by measuring the absorbance of the solutions at 260 nm with a Zeiss PMQ 3 spectrophotometer. To allow an accurate conversion here of $A_{260}$/mL to mg/mL, we have determined the value for the extinction coefficient $E_{260}^{1\text{mg/mL}}$ as described under Results.

*Solvent Viscosity.* For the reductions to $s_{20,w}$ and $D_{20,w}$, the kinematic viscosity of the solvent was determined with a Cannon–Ubbelohde viscosimeter at a temperature of 25 °C, kept constant within 0.01 °C. Solvent density, measured during the determinations of $(\partial\rho/\partial c)_\mu^0$, was used to convert the kinematic to the relative viscosity.

## Results

*Sedimentation Coefficients.* Analytical boundary sedimentation of unfixed and fixed ribosomes from cryptobiotic embryos and nauplii of *Artemia salina* in our standard buffer showed no aggregation or dissociation. Comparison of different kinds of ribosomes in the same run by the use of a four- or six-hole rotor indicated no difference in sedimentation velocity between ribosomes isolated from *A. salina* in different stages of development or between nauplii ribosomes and ribosomes which are all attached to messenger RNA. The pressure-dependent equilibrium between the ribosome and its large and small subunit (Baierlein and Infante, 1974), or an anomolous dependence of sedimentation velocity on the rotor speed as observed for the association complex of *E. coli* ribosomal subparticles (Shcherbukin and Guermant, 1975), can cause an underestimation of the standard sedimentation coefficient. The following results, however, exclude this possibility in our case.

To lower the equilibrium constant, part of a pure ribosome solution in our standard buffer was dialyzed against solvent containing only 2 mM, instead of 9 mM, magnesium acetate; the comparison of both solutions showed no difference in sedimentation behavior. Earlier, we reported our first direct observations by light scattering of the pressure-induced dissociation of *A. salina* ribosomes (Nieuwenhuysen et al., 1975); our further high-pressure light-scattering study (Nieuwenhuysen et al., 1978b; Nieuwenhuysen, Heremans, and Clauwaert, submitted for publication) showed that the influence of the pressures, generated in the analytical ultracentrifuge cell, on the sedimentation behavior of the ribosomes can be neglected in our experimental conditions. Fixation of the ribosomes, which blocks the dynamical equilibrium, did not influence their sedimentation behavior either. No rotor-speed dependence was observed between 12 000 and 36 000 rpm. So finally, we feel confident assigning a value of 81 ± 1 S to the standard sedimentation coefficient $s^\circ_{20,w}$ of unfixed and fixed ribosomes from cryptobiotic embryos and nauplii of *A. salina*. For other eukaryotic cytoplasmic ribosomes, standard sedimentation coefficients have been reported spread around this value (Van Holde and Hill, 1974).

*Diffusion Coefficients.* Single-clipped photon-count autocorrelation spectroscopy of the light, scattered from essentially monodisperse solutions of different kinds of ribosomes, yields their diffusion coefficient. The $Q$ factors (Pusey et al., 1974; Nieuwenhuysen and Clauwaert, 1978a; Nieuwenhuysen, 1978), which reflect eventual deviations from monoexponential decay of the measured correlation functions, were equal to zero to within experimental error ($|Q| < 0.02$). Variation of the sample time $T$ from its optimal value, so that spectra spanned from only 0.5 to more than 5 decay times, did not change the obtained diffusion coefficient $D$.

Figure 1 shows that the correlation spectra of the light, scattered at different angles, yielded the same $D$. The diffusion coefficient of fixed ribosomes from cryptobiotic embryos of *A. salina* was also measured at higher temperatures: reduced to 20 °C, the value remained constant up to 50 °C; further heating up of the solution caused denaturation and aggrega-

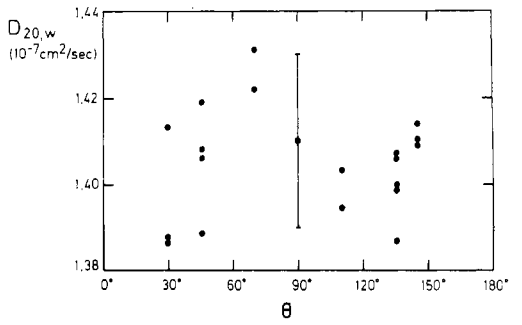


FIGURE 1: Measured values for the standard diffusion coefficient, $D_{20,w}$, of ribosomes from cryptobiotic embryos of *A. salina* as a function of scattering angle $\theta$. (Each point is derived from the weighted fit of the logarithm of a normalized correlation function to a quadratic curve. The error bar is obtained from a series of measurements at 90°.)

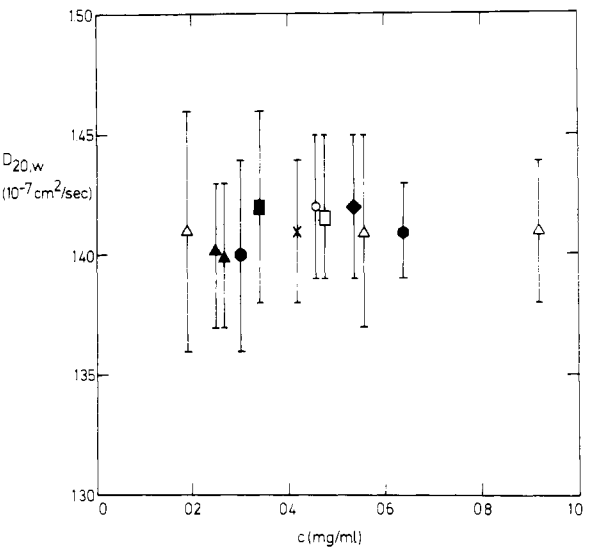


FIGURE 2: Measured values of the standard diffusion coefficient $D_{20,w}$ as a function of ribosome concentration $c$. (Different symbols represent different preparations.) Standard spectrometer: (●, ■, ▲, ♦) unfixed ribosomes isolated from cryptobiotic embryos; (○, □) fixed ribosomes from cryptobiotic embryos and (△) from nauplii. Modified spectrometer: (×, ●) unfixed ribosomes from cryptobiotic embryos of *A. salina*.

tion, which was reflected by a decreasing $D_{20}$ and an increase of the intensity of the scattered light. All these results justify the confidence in our measured values for the standard diffusion coefficient, $D_{20,w}$, for different samples in function of the concentration. No concentration dependence is evident below 1 mg/mL in standard buffer; this is in agreement with theoretical expectations (Pusey, 1974) and with experimental results for *E. coli* ribosomal particles (Koppel, 1974). Figure 2 also shows that the same value for the standard diffusion coefficient was obtained with different experimental setups in different laboratories. Finally, we conclude from our data that the ribosomes isolated from cryptobiotic embryos and nauplii of *A. salina* have the same standard diffusion coefficient at infinite dilution, which is not altered by fixation: $D^\circ_{20,w} = (1.41 \pm 0.02) \times 10^{-7}$ cm$^2$/s. Note that we also found the same standard sedimentation coefficient for the different kinds of ribosomes.

*Extinction Coefficient, Density Increment, and Partial Specific Volume.* The extinction coefficient $E_{260}{}^{1\,mg/mL}$ of the ribosomes from the cryptobiotic embryos has been obtained from the relation between the absorbance at 260 nm of a ribosome solution and its RNA and protein content. By determining the phosphorus content we have found that 1 $A_{260}$ unit contains 41.6 ± 1.0 μg of RNA, and by using the original Lowry method and a modified version, with BSA as standard, we have found that 1 $A_{260}$ unit contains 42.4 ± 1.6 μg of protein (Nieuwenhuysen et al., 1978a). This value for the protein content is somewhat uncertain because the color yield in the Lowry method differs for BSA and ribosomal proteins and because it can be influenced by the presence of RNA; however, both effects cancel each other to a great extent (Sherton and Wool, 1974). We have also used the biuret method for which the color yield is independent of the proteins involved (Sacchi et al., 1977). By adding ribosomal RNA in equimolar amounts to the standard BSA solutions, we checked that its presence does not interfere with the color yield. From three series of measurements on three different preparations of ribosomes, we conclude that 1 $A_{260}$ unit contains 41 ± 2.5 μg of protein. Furthermore, we have determined by analytical equilibrium centrifugation that the buoyant density $\theta$ of the formaldehyde-fixed ribosomes in a CsCl density gradient equals 1.570 ± 0.005 g/cm$^3$, from which we can calculate a protein content $p$ of 51% (Nieuwenhuysen et al., 1978a), using the empirical relation (Hamilton, 1971):

$$1/\theta = [(100 - p)0.53 + p0.74]/100$$

The validity of this method has recently been confirmed (Sacchi et al., 1977). All these data are in excellent agreement and they give us $E_{260}{}^{1\,mg/mL} = 12.0 \pm 0.4$. This value is also consistent with the known $E_{260}{}^{1\,mg/mL}$ of ribosomal RNA as we have shown elsewhere (Nieuwenhuysen et al., 1978a).

Two series of measurements with independent preparations yielded plots of the difference between the density of the ribosome solutions and of the dialysate, against the absorbance at 260 nm of the solutions. These show that $(\partial\rho/\partial c)_\mu$ is independent of the concentration in the studied range (<60 $A_{260}$/mL) and yield $(\partial\rho/\partial c)_\mu{}^0 = 0.0312 \pm 0.0003$ mg/$A_{260}$. Using here our value for the extinction coefficient, we obtain $(\partial\rho/\partial c)_\mu{}^0 = 0.37 \pm 0.01$. With the equation

$$(\partial\rho/\partial c)_\mu{}^0 = 1 - \bar{v}^0\rho^0$$

we find for the partial specific volume at infinite dilution of the ribosomes $\bar{v}^0 = 0.63 \pm 0.01$ cm$^3$/g.

Our $\bar{v}^0$ is somewhat lower but within experimental error equal to the reciprocal of our measured value for the buoyant density of the ribosomes in CsCl. This is in complete accordance with results for yeast ribosomes (Mazelis and Petermann, 1973).

*Molecular Weight.* Substitution of our measured values for $s^\circ_{20,w}$, $D^\circ_{20,w}$, and $(\partial\rho/\partial c)_\mu{}^0$ in (Eisenberg, 1976)

$$M = \frac{s^\circ_{20,w} RT}{D^\circ_{20,w} \left(\frac{\partial\rho}{\partial c}\right)_\mu^0}$$

where $R$ is the gas constant and $T = 293$ K, yields a molecular weight $M = (3.8 \pm 0.1) \times 10^6$ for the ribosomes from the cryptobiotic embryos.

Analytical CsCl density gradient equilibrium sedimentation experiments of ribosomes isolated from cryptobiotic embryos and nauplii showed no difference between their buoyant density. This indicates that their density increment will be equal to within experimental error. So, together with our results for $s^\circ_{20,w}$ and $D^\circ_{20,w}$, this also shows that their molecular weight is the same to within experimental error.

For other eukaryotic ribosomes, molecular weights between 3.3 and 5.2 × 10$^6$ have been determined with other methods, which are not free of criticism (Van Holde and Hill, 1974).

*Hydrodynamic and Dry Volume and Radius.* Substitution of our measured value for $D^\circ$ of *A. salina* ribosomes in the

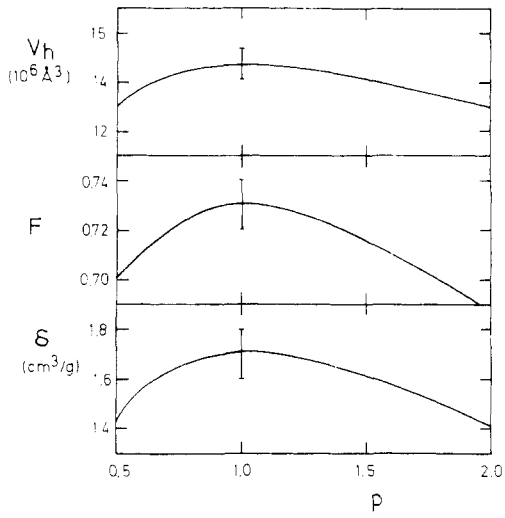


FIGURE 3: Combinations of the elongation $p$ of an ellipsoid of revolution with, respectively, the hydrodynamic volume $V_h$, the ratio of solvated to dry volume $F$, and the volume of solvent per unit weight of dry macromolecule $\delta$, which are consistent with the measured $s°$, $D°$, and $\bar{v}°$ of *Artemia salina* ribosomes.

Stokes–Einstein equation

$$R_h = \frac{kT}{6\pi\eta^0 D^0}$$

where $k$ is Boltzmann's constant, $T$ the absolute temperature, and $\eta^0$ the solvent viscosity, gives us the radius of the hydrodynamically equivalent sphere (Koppel, 1974), $R_h = 152 \pm 2$ Å. Due to deviations of the ribosome shape from a sphere, their real hydrodynamic volume $V_h$ will be smaller than that of the hydrodynamically equivalent sphere

$$V_{h,max} = \frac{4\pi}{3}\left(\frac{kT}{6\pi\eta^0 D^0}\right)^3$$

Taking an ellipsoid of revolution as a model, we have

$$V_h = V_{h,max} t^3(p)$$

where $t(p) \leqslant 1$, a tabulated function of the elongation $p$ (Sadron, 1953). In Figure 3, we plotted the combinations of $p$ and $V_h$, starting from $V_{h,max} = (14.7 \pm 0.6) \times 10^6$ Å$^3$, which are consistent with our measured value of $D^0$.

For any macromolecule, we can define the dry volume

$$V_d = \frac{\bar{v}^0 M}{N}$$

where $N$ is Avogadro's number. Substitution of our values for $\bar{v}^0$ and $M$ yields $V_d = (4.0 \pm 0.2) \times 10^6$ Å$^3$ for *A. salina* ribosomes. This volume can be packed into a sphere with radius

$$R_d = \left(\frac{3\bar{v}^0 M}{4\pi N}\right)^{1/3}$$

This dry radius has the value $R_d = 98 \pm 2$ Å.

*Solvation.* We can define two solvation parameters: the fraction of the volume of the solvated macromolecule which is occupied by solvent

$$F = 1 - \frac{V_d}{V_h}$$

and the volume of solvent per unit weight of dry macromolecule

$$\delta = \frac{V_h - V_d}{M/N}$$

In Figure 3, we plotted combinations of the elongation $p$ of an ellipsoid of revolution and $F$ or $\delta$, starting from $F_{max} = 0.73 \pm 0.01$ and $\delta_{max} = 1.7 \pm 0.1$ cm$^3$/g at $p = 1$, which are consistent with our measured values $s°_{20,w} = 81$ S, $D°_{20,w} = 1.41 \times 10^{-7}$ cm$^2$/s and $\bar{v}^0 = 0.63$ cm$^3$/g of *A. salina* ribosomes.

## Discussion

Our data do not indicate large physical changes in the ribosomes during development and concomitant cell differentiation in *A. salina*. Up to now, two-dimensional gel electrophoresis did not reveal any change either in the ribosomal proteins of *A. salina* (Möller et al., 1975).

It has been reported that the polysomal ribosomes from chick-embryo muscle tissue sediment 9% more rapidly than the free, single ribosomes (Vournakis and Rich, 1971). Such a difference would be well beyond our experimental error, but we did not find it for *A. salina* ribosomes. Thus, we believe that the more compact form of ribosomes attached to messenger RNA (Vournakis and Rich, 1971) should not be generalized. It should be noted that the chick-embryo ribosomes had a high buoyant density in CsCl (1.60–1.61 g/cm$^3$), which probably reflects the high salt content of their solvent (250 mM KCl) and the release of some proteins: their molecular weight, however, was calculated to be the largest that has been reported for ribosomes as far as we know ($5.2 \times 10^6$), which is more than two times that of *E. coli* ribosomes (Van Holde and Hill, 1974; Koppel, 1974); furthermore, we calculated that their solvation would be twice as large as for *A. salina* ribosomes. We should mention here already that the attachment of the ribosomes to mRNA fragments influences their pressure dependence, as revealed by light scattering (Nieuwenhuysen et al., 1978b; Nieuwenhuysen, Heremans and Clauwaert, submitted for publication).

The experimental value that we received for the standard diffusion coefficient of *A. salina* ribosomes is considerably higher than those reported without experimental details for ribosomes isolated from chick-embryo muscle tissue ($1.04$–$1.14 \times 10^{-7}$ cm$^2$/s) (Vournakis and Rich, 1971); however, our comment on this study in the previous paragraph should be borne in mind.

We can consider our values for the diffusion coefficient and the hydrodynamic size of *A. salina* ribosomes in the light of results from X-ray scattering experiments, which yielded 370 and 270 Å for the maximal dimensions of rat-liver ribosomes (Damaschun et al., 1974). We calculated the value $15.2 \times 10^6$ Å$^3$ for the maximal rat-liver ribosome volume, i.e., the volume of a prolate ellipsoid of revolution with axes of 370 and 270 Å and thus with an elongation $p = 1.32$. The prolate ellipsoid of revolution, with the same elongation, which is consistent with our value for the diffusion coefficient of *A. salina* ribosomes, has a volume of $(14.4 \pm 0.6) \times 10^6$ Å$^3$. A closer agreement between the two volumes could not be expected.

The hydrodynamic volume and the two solvation parameters will probably be equal to, respectively, $V_{h,max}$, $F_{max}$, and $\delta_{max}$ to within experimental error, as can be seen in Figure 3.

The results in this paper form a sound basis for the interpretation of the data from neutron-scattering experiments, performed at the Institut Von Laue-Langevin in Grenoble, on these ribosomes in the same solvent.

In Table I, we summarize out results, together with the corresponding published data for ribosomes from the prokaryote *Escherichia cole*. A comparison of both series of data shows how much the ribosome has increased in size during evolution, without gross changes in its "spongelike" structure, as reflected by the respective values of $F$, the large fraction of their volume that is occupied by solvent.

TABLE I: Comparison between Physical Properties of Ribosomes Isolated from *Escherichia coli* (Prokaryotic) and *Artemia salina* (Eukaryotic).

| | *E. coli*[a] | *A. salina* |
|---|---|---|
| $s^\circ_{20,w}$ (S) | 70 | 81 |
| $D^\circ_{20,w}$ ($10^{-7}$ cm$^2$/s) | 1.71 | 1.41 |
| $\bar{v}^0$ (cm$^3$/g) | 0.60 | 0.63 |
| $M$ ($10^6$) | 2.5 | 3.8 |
| $R_h$ (Å) | 126 | 152 |
| $V_{h,max}$ ($10^6$ Å$^3$) | 8.4 | 14.7 |
| $V_d$ ($10^6$ Å$^3$) | 2.5 | 4.0 |
| $R_d$ (Å) | 84 | 98 |
| $R_h/R_d$ | 1.50 | 1.55 |
| $F_{max}$ | 0.70 | 0.73 |
| $\delta_{max}$ (cm$^3$/g) | 1.4 | 1.7 |

[a]Cited and calculated from Koppel (1974) and references cited therein.

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References

Baierlein, R., and Infante, A. A. (1974), *Methods Enzymol. 30,* 328.

Damaschun, G., Müller, J. J., Bielka, H., and Böttger, M. (1974), *Acta Biol. Med. Ger. 33,* 817.

Dubin, S. B., Clark, N. A., and Benedek, G. B. (1971), *J. Chem. Phys. 54,* 5158.

Eisenberg, H. (1976), Biological Macromolecules and Polyelectrolytes in Solution, Oxford, Clarendon Press.

Foord, R., Jones, R., Oliver, C. J., and Pike, E. R. (1969), *Appl. Opt. 8,* 1975.

Foord, R., Jakeman, E., Oliver, C. J., Pike, E. R., Blagrove, R. J., Wood, E., and Peacocke, A. R. (1970), *Nature (London) 227,* 242.

Hamilton, M. G. (1971), *Methods Enzymol. 20,* 512.

Hentschel, C., and Tata, J. R. (1976), *Trends Biochem. Sci. 1,* 97.

Hughes, A. J., Jakeman, E., Oliver, C. J., and Pike, E. R. (1973), *J. Phys. A: Math. Nucl., Gen. 6,* 1327.

Hultin, T., and Morris, J. E. (1968), *Dev. Biol. 17,* 143.

Jolly, D., and Eisenberg, H. (1976), *Biopolymers 15,* 61.

Koppel, D. E. (1974), *Biochemistry 13,* 2712.

Kratky, O., Leopold, H., and Stabinger, H. (1973), *Methods Enzymol. 27,* 98.

Mazelis, A. G., and Petermann, M. L. (1973), *Biochim. Biophys. Acta 312,* 111.

Möller, W., Slobin, L. I., Amons, R., and Richter, D. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 4744.

Nieuwenhuysen, P. (1978), *Macromolecules 11,* 832.

Nieuwenhuysen, P., and Clauwaert, J. (1977), *Rev. Sci. Instrum. 48,* 699.

Nieuwenhuysen, P., and Clauwaert, J. (1978a), *J. Polym. Sci., Polym. Symp.* (in press).

Nieuwenhuysen, P., and Clauwaert, J. (1978b), *Biopolymers* (in press).

Nieuwenhuysen, P., and Slegers, H. (1978), in Collection of Abstracts, International Symposium on Biomolecular Structure, Conformation, Function, and Evolution, University of Madras, India, p 106; *Anal. Biochem.* (in press).

Nieuwenhuysen, P., Clauwaert, J., and Heremans, K. (1975), *Arch. Int. Physiol. Biochim. 82,* 972.

Nieuwenhuysen, P., De Herdt, E., and Clauwaert, J. (1978a), in Collection of Abstracts, International Symposium on Biomolecular Structure, Conformation, Function, and Evolution, University of Madras, India, p 107; *Anal. Biochem. 88,* 532.

Nieuwenhuysen, P., Heremans, K., and Clauwaert, J. (1978b), *Arch. Int. Physiol. Biochim.* (in press).

Pusey, P. N. (1974), in Photon Correlation and Light Beating Spectroscopy, Cummins, H. Z., and Pike, E. R., Ed., New York, N.Y., Plenum Press, p 387.

Pusey, P. N., Koppel, D. E., Schaefer, D. W., Camerini-Otero, R. D., and Koenig, S. H. (1974), *Biochemistry 13,* 952.

Sacchi, A., Ferrini, V., Londei, P., Cammarano, P., and Maraldi, N. (1977), *Biochem. J. 168,* 245.

Sadron, C. (1953), *Prog. Biophys. Mol. Biol. 3,* 237.

Shcherbukhin, V. V., and Guermant, C. (1975), *Biophys. Chem. 3,* 21.

Sherton, C. C., and Wool, I. G. (1974), *Mol. Gen. Genet. 135,* 97.

Van Holde, K. E., and Hill, W. E. (1974) *Ribosomes, Meet., 1973,* 53.

Vournakis, J., and Rich, A. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 3021.