The molecular weight of *Artemia* ribosomes, as determined from their refractive-index increment and light-scattering intensity

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Cytoplasmic ribosomes were isolated from the cryptobiotic embryos of the brine shrimp *Artemia salina*. Measurements of their refractive-index increments and light-scattering intensities give a value for their molecular weight of $(3.4 \pm 0.2) \times 10^6$.

While the details of the structure and function of *Escherichia coli* ribosomes are being unravelled, progress is also being made in studies of the larger ribosomes of various eukaryotes (Chambliss *et al.*, 1980). However, a critical examination of published data shows that the basic physicochemical properties of eukaryotic ribosomes still remain to be firmly established (Nieuwenhuysen & Clauwaert, 1980). In the present paper we focus on the molecular weight of cytoplasmic eukaryotic ribosomes, as determined from their refractive-index increments and light-scattering intensities. The brine shrimp *Artemia salina* (Persoone *et al.*, 1980) was used as a source of eukaryotic cytoplasmic ribosomes, for the following reasons. The seed-like desiccated cryptobiotic undeveloped embryos (cysts) are available in large quantities and they can be easily stored. These cysts contain a large supply of free unprogrammed ribosomes that are devoid of mRNA, very few membrane-associated ribosomal particles, and no detectable polyribosomes (Hultin & Morris, 1968). The unprogrammed ribosomes of the cysts are particularly useful for translation of exogenous mRNA *in vitro* (see, e.g., Kramer & Hardesty, 1980). *Artemia* is widely used to study the role of protein biosynthesis in executing the development programme (see, e.g., Bagshaw & Warner, 1979). Ribosomal particles from *Artemia* are also used as models for other eukaryotic ribosomes in structural studies by electron microscopy (see, e.g., Boublik & Hellmann, 1978). The proteins from ribosomes of *Artemia* (and wheat germ) may be the first eukaryotic ribosomal proteins to be individually purified in amounts sufficiently large for extensive characterization (Ting Shih *et al.*, 1979).

A preliminary report of this work has been published (Nieuwenhuysen *et al.*, 1980).

Experimental

Preparation of ribosome solutions

Ribosomes were isolated from undeveloped

cryptobiotic embryos (cysts) of *Artemia salina* from San Francisco Bay. In all steps of the preparation and during measurement, the ribosome solutions contained 20 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/KOH buffer (pH 7.5), 1 mM-dithiothreitol, 70 mM- or 100 mM-KCl and 9 mM-magnesium acetate. The cysts were washed and ground, and postmitochondrial supernatant was prepared from the filtered homogenate by differential pelleting (Nieuwenhuysen & Clauwaert, 1980). For laser-light-scattering, the preparation was continued as follows (Nieuwenhuysen & Slegers, 1978a,b): overnight ultracentrifugation of the postmitochondrial supernatant in a high-density sucrose gradient, precipitation of the ribosomes from the top fractions with poly(ethylene glycol) 6000, rate-zonal sucrose-density-gradient sedimentation of the resuspended ribosomes, and dialysis of the top fractions before measurement. For all other experiments, centrifugation in a high-density sucrose gradient and precipitation were replaced by overnight centrifugation of the postmitochondrial supernatant in a Spinco Ti 60 rotor at 50 000 rev./min through a 20 ml pad of 50% (w/w) sucrose at 4°C; this yielded clear colourless ribosome pellets (Nieuwenhuysen & Clauwaert, 1980).

The purity of the ribosome solutions was checked by absorbance spectroscopy, electron microscopy after negative staining, isopycnic sedimentation in a CsCl density gradient in an analytical and in a preparative ultracentrifuge, photon-correlation spectroscopy of scattered laser light, rate-zonal centrifugation in sucrose density gradients and analytical boundary sedimentation in a Beckman model E or in an MSE ultracentrifuge (Nieuwenhuysen & Slegers, 1978a,b; Nieuwenhuysen & Clauwaert, 1978, 1980).

Subunits were prepared as described elsewhere (Nieuwenhuysen & Clauwaert, 1980).

Determination of ribosome concentration

Ribosome concentrations were determined from

absorbance measurements at 260 nm on appropriately diluted solutions in 1 cm quartz cuvettes, in a Zeiss PMQ 2 or 3 spectrophotometer. The absorbance at 260 nm of a 1 mg/ml solution was obtained from the amount of protein (Nieuwenhuysen & Clauwaert, 1978, 1980) and RNA (see next paragraph) per A_{260} unit of ribosomes.

The RNA content of ribosome solutions was determined from their phosphorus content (Ames, 1966). By taking into account the base composition of the ribosomal subunits (Vandenberghé *et al.*, 1980), it was accepted that the phosphorus content of the free acid form of RNA in the large and the small ribosomal subunits is 9.62 and 9.64% by weight respectively. Solutions of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ were used as standards in the phosphorus determinations; their concentration was measured accurately by acid-base titration before each series of determinations. Special care was taken to clean all glassware carefully by immersion in fresh chromic acid for at least 24 h, followed by extensive washing with twice-distilled water and steam.

Measurement of refractive-index increments

Ribosome solutions with concentrations below 3 mg/ml were brought to thermodynamic equilibrium of diffusible components by exhaustive dialysis. They were centrifuged at low speed before measurement. Differences in the refractive index (Δn) of the solution and the outer diffusate were measured at 436 and 546 nm with a Rayleigh-Haber-Löwe interferometer (Bauer *et al.*, 1960). This instrument requires no calibration; its performance was checked with solutions of dried KCl. The refractive-index increments at zero concentration and constant chemical potential μ of all diffusible components, $[\partial n(\lambda_0)/\partial c]_\mu^0$, were then obtained from a plot of Δn against concentration c .

Light-scattering

First series of experiments. Total intensity measurements of scattered laser light were performed with a modified Malvern equipment (Jolly & Eisenberg, 1976). The light-source was an intensity-stabilized Spectra Physics argon ion laser, emitting light at $\lambda_0 = 515.5$ nm. The index-matching bath was filled with toluene at 20.0°C. A benzene sample (Godfrey & Eisenberg, 1976) was used as a calibration standard (Voordouw *et al.*, 1978). The cylindrical scattering cells (Godfrey & Eisenberg, 1976) were painstakingly cleaned with a flow of filtered twice-distilled water (Dubin *et al.*, 1971). Corrections for dead-time effects in the photo-multiplier circuitry (Pike *et al.*, 1975) were negligible.

Second series of experiments. Light-scattering intensities were also measured with a Brice-Phoenix model 5200 photometer, manufactured by Wood

Mfg. Co., with polarized and unpolarized light with λ_0 of 436 and 546 nm. This particular apparatus has been calibrated especially for molecular-weight determinations of macromolecules in aqueous solutions (De Voegt *et al.*, 1979). Special high-quality conical cells (Hellma) were used to avoid reflexions. They were cleaned by flushing them with condensing acetone vapour in a reflux system (Tabor, 1972). The filled cells could be centrifuged in a adapter at 6000 rev./min.

Preparation of dust-free solutions. Filtration of ribosome solutions as described for DNA (Godfrey & Eisenberg, 1976) with Millipore and/or Nuclepore filters was not satisfactory. Instead, the solutions were clarified by centrifugation; the material from the middle of the tube was withdrawn carefully.

Data analysis. The following equation was used to determine the molecular weight M (e.g., Voordouw *et al.*, 1978):

$$[Kc/\Delta R(\lambda_0, \theta)] \cdot P(\lambda_0, \theta) = (1/M + 2A_2c + \dots)$$

where c is the concentration in g/ml, A_2 the second virial coefficient and $P(\lambda_0, \theta)$ the intraparticle interference factor at wavelength λ_0 and scattering angle θ . K is proportional to $n^2[(\partial n(\lambda_0)/\partial c)_\mu^0]^2/\lambda_0^4$, and ΔR is determined from the excess scattered intensities, corrected for depolarization (Utiyama, 1972), by introducing the Cabannes factor (Champetiers, 1972).

Results and discussion

Relation between absorbance and concentration

From phosphorus determinations it was found that the large and the small subunits of *Artemia* ribosomes contain 40.8 (± 1) μg of RNA per A_{260} unit. It is satisfactory that the same value has been found for particles with different ratios of RNA to protein content, because the contribution of the proteins to the absorbance at 260 nm is negligible (Araco *et al.*, 1975; Elson *et al.*, 1979). The same value can therefore be used for the complete ribosomes also.

Different colorimetric determinations have shown that the ribosomes contain 42 (± 2) μg of protein per A_{260} unit (Nieuwenhuysen & Clauwaert, 1978, 1980). Therefore they contain 49% by weight of RNA.

From the data above, it was calculated that a 1 mg/ml solution of *Artemia* ribosomes has an absorbance at 260 nm of 12.1 ± 0.4 .

Refractive-index increments

From our measurements of the increase in refractive index as a function of absorbance, and from the relationship between absorbance and concentration in mg/ml (determined as described above), we have obtained values for $[\partial n(\lambda_0)/\partial c]_\mu^0$ at

546 and 436 nm of $0.168 (\pm 0.009)$ and $0.176 (\pm 0.009)$ ml/g. These data are depicted in Fig. 1, together with analogous published data for other nucleoproteins, nucleic acids and proteins. From this Figure it seems that our values for $[\partial n(\lambda_0)/\partial c]_{\mu}^0$ and their wavelength dispersion are very reasonable. The values for ribosomal particles from *E. coli* (Fig. 1) appear to be somewhat too high; this may at least partly explain the low values for their molecular weight, which were derived from light-scattering intensities combined with these data (Igarashi *et al.*, 1973). The earlier and less accurate value of about

0.20 ml/g quoted for *E. coli* ribosomal particles (Scafati *et al.*, 1971) seems much too high; this value has been accepted, however, in a recent estimate by laser-light-scattering of the molecular weight of ribosomal subunits from wheat germ (Sperrazza *et al.*, 1980).

Light-scattering intensities

In one of the test experiments the intensity was measured of laser light scattered by a solution, containing $4.3 A_{260}$ /ml, of small ribosomal subunits isolated from the prokaryote *E. coli*. These were prepared in the laboratory of Dr. Spitnik-Elson (Spitnik-Elson *et al.*, 1978). For their absorbance at 260 nm, the value 14.4 ml/mg was accepted, which is the average of the published values 14.8 (Hill *et al.*, 1969) and 14.0 (Igarashi *et al.*, 1973) ml/mg. For their refractive-index increment at 515.5 nm, a value of 0.171 ml/g was derived from Fig. 1. Variations of light-scattering intensity with scattering angle ($30^\circ < \theta < 140^\circ$) larger than experimental error were not observed; this is satisfactory, since the ribosomal subunits are much smaller than the wavelength of light. By neglecting an eventual small concentration-dependence, a value for their molecular weight was calculated to be 0.84×10^6 ; the uncertainty due to intensity and absorbance measurements is about 3%. This result lies between some of the values reported previously: 0.90×10^6 (Hill *et al.*, 1969), 0.78×10^6 (Igarashi *et al.*, 1973) and 0.87×10^6 (Koppel, 1974).

From the radius of gyration of *Artemia* ribosomes (Nieuwenhuysen, 1978), values for their scattering factors $P(\lambda_0, 90^\circ)$ were calculated; these are close to 0.99. Their depolarization ratio at 90° for unpolarized incident light was 0.009. By using these data, together with the measured refractive-index increments (see above) and intensities of scattered light, we have obtained values for $[Kc/\Delta R] \cdot P$ at $\theta = 90^\circ$ that are plotted as a function of the concentration c in Fig. 2. These results agree remarkably well, in view of the large differences in experimental setup, calibration procedures and the nature of the incident light.

Molecular weight of *Artemia* ribosomes

The straight line fitted to the points in Fig. 2 gives an intercept corresponding to a value of $(3.4 \pm 0.2) \times 10^6$ for the molecular weight of *Artemia* ribosomes.

For comparison, the molecular weight of the ribosomes can be estimated roughly by combining published estimates for the molecular weight of their RNA components with our measured ratio of their RNA to protein content. Cammarano *et al.* (1975) have determined the apparent molecular weight of ribosomal RNA from various eukaryotes by gel electrophoresis in formamide, with *E. coli* rRNA as

Fig. 1. Refractive-index increments at wavelengths λ_0 , $[\partial n(\lambda_0)/\partial c]_{\mu}^0$, of nucleoproteins, as a function of their RNA or DNA content

a, *Artemia* ribosomes (present work); b, *E. coli* ribosomes and ribosome subunits (Igarashi *et al.*, 1973); c, bacteriophage R17; d, bacteriophage T7 (Camerini-Otero *et al.*, 1974); e, complex reconstituted by saturating plasmid DNA with acid-extracted calf thymus histones (G. Voordouw, Z. Kam, N. Borochoy & H. Eisenberg, unpublished work); f, filamentous bacteriophage fd (Berkowitz & Day, 1980). ● and ○, RNA content; ■ and □, DNA content. ○ and □, $\lambda_0 = 436$ nm; ● and ■, $\lambda_0 = 546$ nm. The symbols at 0% nucleic acid indicate mean values of the refractive-index increment and its wavelength dispersion for proteins, based on a compilation of published data (Timasheff, 1970). At 100% nucleic acid values are indicated for native RNA of bacteriophage MS 2 (Slegers *et al.*, 1973), for DNA in 0.2 M-NaCl (Jolly & Eisenberg, 1976) and for DNA of bacteriophage fd (Day & Berkowitz, 1977).

Fig. 2. Light-scattering intensity of *Artemia* ribosomes as a function of their concentration c

The error bars on the values obtained with vertically polarized laser light with λ_0 515.5 nm (■) indicate the uncertainty due to intensity and absorbance measurements. The error bars on the values obtained with incoherent light (●) indicate the spread among results obtained with different calibration methods (measurements relative to the intensity of the incoming beam and to the intensity scattered by benzene) and with different kinds of light (polarized with λ_0 546 nm, and unpolarized with λ_0 436 and 546 nm).

standards; the values given for the large and the small rRNA from *Artemia* are 1.38×10^6 and 0.65×10^6 ; in the case of chick, rat liver and HeLa cells, these values are 1.60×10^6 and 0.65×10^6 . Spohr *et al.* (1976) have determined the molecular weight ($\pm 10\%$) of various animal RNA species by gel electrophoresis in formamide at an elevated running temperature to obtain complete denaturation. They too used *E. coli* RNA as standards and obtained a smaller value for the small rRNA from duck and HeLa cells, namely 0.605×10^6 , and for the large rRNA 1.30×10^6 and 1.75×10^6 respectively. These published data indicate that the total molecular weight of the RNA in *Artemia* ribosomes may be around 2.1×10^6 or 1.9×10^6 . Combining this with the RNA content of the ribosomes (49% by weight, see above) would then yield a molecular weight of the ribosome between about 3.8×10^6 and 4.2×10^6 . The large uncertainty here is typical of the lack of conclusive data on the physicochemical properties of eukaryotic ribosomes (Nieuwenhuysen & Clauwaert, 1980). In view of their size and complexity, it is unlikely that a more reliable and accurate estimate of their molecular weight will be possible in the near future.

This work on an absolute scale indicates that light-scattering will be useful for relative measurements comparing the mass of ribosomes from various eukaryotes.

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Note added in proof (received 27 April 1981)

After we had submitted this paper for publication, an abstract has been published of a study of the ribosomal RNA species from *Artemia* (Cruces *et al.*, 1981). They report results indicating lower molecular-weight values than those given by Cammarano *et al.* (1975) cited above. Thus the recent values agree somewhat better with our results.

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