Physicochemical, Chemical, and Biological Studies on Wheat Embryo Ribosomes*

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ABSTRACT: Wheat embryo ribosomes with a nucleic acid protein content of 55 and 45%, respectively, have been isolated and subjected to a detailed physicochemical investigation using the techniques of sedimentation velocity, diffusion, viscometry, pycnometry, ultraviolet absorption, and optical rotatory dispersion. The intrinsic sedimentation constant, determined by both schlieren and ultraviolet optics, is 80 S and the $D^0_{20,w}$ value, evaluated through the use of the Möller method, is 1.15×10^{-7} cm²/sec. The molecular weight of the particle was estimated from the Svedberg equation as $4.25 (\pm 0.5) \times 10^6$, and it contains approximately 80% of its enclosed volume as water of hydration. Three ultracentrifugally identifiable components with $s^0_{20,w}$ values of 60, 40, and 26 S, respectively, are obtained as dissociation products when magnesium is removed from the ribosomes, and in this respect this system behaves similarly to ribosomes from other plant sources. Optical rotatory dispersion studies on wheat embryo ribosomes and their constituent ribonucleic acid (RNA) and proteins indicate that the ribosomal proteins are similar to protamines in that they have little α-helical content, and that the conformation of RNA and proteins inside the ribosome is very similar to that in the free state. In agreement with reports by other workers, an amino acid incorporating system may be obtained from wheat embryos. This system has very low endogenous activity, but phenylalanine incorporation can be stimulated by the addition of polyuridylate.

Lyttleton (1960) first described the preparation of ribosomal particles from wheat embryo. Marcus and Feeley (1964) observed that wheat embryo ribosomes were active in protein synthesis when artificial mRNA was added to the incorporating medium. Allende and Bravo (1966) expanded the bioassay work of Marcus and Feeley and in particular examined the effect of supernatant factor proteins from different cells on the protein-synthesizing ability of wheat embryo ribosomes. Chen *et al.* (1966) and DeGroot *et al.* (1967) reported findings similar to those of Allende and Bravo.

Since commercially prepared wheat embryo is relatively inexpensive and may be stored for long periods of time in the cold with little loss of its biologically active components, it is a convenient source for large-scale preparation of ribosomes for the study of protein synthesis *in vitro*.

The experiments reported herein describe a detailed physicochemical characterization of wheat embryo ribosomes. Measurements of sedimentation, diffusion, viscosity, partial specific volume, optical rotatory dispersion, ultraviolet absorption, chemical analysis, and bioassay on wheat embryo ribosomes are presented and compared with the corresponding properties of other ribosome systems. The physical properties of ribosomes from many other sources have been well documented in treatises by Petermann (1964) and Bonner and Varner (1965).

Materials and Methods

Preparation of Ribosomes. Wheat embryo (34 g), commercially prepared from the Thatcher variety of wheat, was homogenized in a Waring Blendor with 500 ml of a buffering medium composed of 0.050 M KCl, 0.025 M Tris-HCl (pH 7.5), 0.005 M $MgCl_2$, and 0.006 M β-mercaptoethanol. This medium was found to be ideal for the reproducible preparation of highly pure ribosomes. After 2 min of homogenization, the homogenate was passed through cheesecloth to remove the still intact embryos as well as some debris. The solution was then centrifuged at 25,000 rpm for 30 min in the no. 30 rotor of the Beckman-Spinco Model L preparative ultracentrifuge to remove most of the heavy contaminants. The transluscent supernatant was transferred to the no. 40 rotor and centrifuged at 38,000 rpm for 90 min to pellet the ribosomes. The supernatant from this step was dialyzed against the preparative buffer medium, and the nondiffusible portion was subsequently used as "supernatant factor" in the incorporation studies. The pelleted ribosomes were dissolved in the buffered medium and diluted to a final concentration of 0.1%. This solution was centrifuged in the no. 40 rotor at 22,000 rpm for 30 min to remove most of the polysomes. The precipitate was discarded and the supernatant was centrifuged again in the no. 40 rotor at 38,000 rpm for 90 min to repellet the ribosomes. The supernatant was discarded and

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the pellets were dissolved in a minimal amount of buffered medium and adjusted by dilution to about 0.5%. This solution was given a final low-speed spin to remove any remaining heavy contaminants and then stored at 0°. All preparative work was carefully conducted at 0°. By using this methodology it was possible to prepare routinely 500 mg of wheat embryo ribosomes in less than 8 hr. Each preparation was examined in the Model E analytical ultracentrifuge and found to be free of extraneous particles.

Ribonucleate Preparations. Wheat embryo rRNA and tRNA were prepared by the methods of Singh and Lane (1964) and Glitz and Dekker (1963), respectively, and were kindly provided by Dr. B. G. Lane and Mr. M. Gray.

Chemical Analyses. Protein was determined by the Oyama and Eagle (1956) modification of the Lowry method, using bovine serum albumin as a standard. The ribosome solutions were first dialyzed free of β-mercaptoethanol, which interferes with the estimations. RNA was determined by the method of Ceriotti (1955), using high molecular weight wheat embryo RNA, as well as ribose as standards. The dry weight of the ribosomes was determined by drying at 100° *in vacuo*. Using the above methodology, the ribosomes were found to contain 55% RNA and 45% protein. Ribosome solutions of varying concentration also obey Beer's Law over an optical density range of 0.100–0.660 and possess an extinction coefficient ($E^{1\%}_{1\text{ cm, }258\text{ m}\mu}$) of 115. This value was used to determine the concentration of ribosomes throughout this study. Absorption measurements were carried out in the Beckman DU spectrophotometer and the Beckman DB-G recording spectrophotometer with a T_m[1] analysis attachment. The rate of temperature increase was 0.7°/min.

Assays for Biological Activity. Bioassay was conducted according to the technique of Allende and Bravo (1966), using an incubation medium containing 50 mM Tris-HCl (pH 7.5), 25 mM KCl, 7.5 mM $MgCl_2$, 0.5 mM GTP (Pabst), 1.0 mM ATP (Pabst), 5 mM creatine phosphate (Calbiochem), 10 μg of creatine phosphokinase (Behringer, Mannheim), 150 μg of wheat embryo tRNA, 300 μg of wheat embryo ribosomes, 40 μg of polyuridylate (Light and Co.), 8 μM [^{14}C]phenylalanine (Schwarz BioResearch), and ~1 mg of "supernatant factor" protein, all in 1 ml of solution. After incubation for 45 min the reaction was stopped by addition of 4 ml of 5% TCA. Casein (1 mg) was added as carrier protein. The precipitate was collected by centrifugation and then boiled for 15 min in 3 ml of 5% TCA. This precipitate was collected by centrifugation and washed three times with 3 ml of cold 5% TCA, once with 1:1 ether–ethanol, and once with ether. When dry, the precipitate was dissolved in 1.00 ml of concentrated formic acid. Exactly 0.50-ml portions of this solution were added to 10 ml of Bray's (1960) solution and counted in a Nuclear-Chicago Model 8260 liquid scintillation counter.

Hydrodynamic Measurements. Sedimentation velocity runs were carried out in a Beckman-Spinco Model E ultracentrifuge equipped with the electronic speed-control system and utilizing both the ultraviolet absorption and the schlieren optical systems. With the ultraviolet system, the sedimentation boundaries were recorded photographically on Kodak blue sensitive M film. Graphical representation of ribosomes as a function of cell position was obtained from the films using a Spinco Analytrol densitometer. All the sedimentation velocity runs were carried out at 40,000 rpm and at temperatures near 5° and were corrected for solvent density and viscosity at 20°.

The intrinsic diffusion constants ($D^0_{20,w}$ values) for the ribosomes were determined by the procedure of Möller (1964) which employs the light absorption method. The diffusion experiment was performed in two phases of the same operation: a 12-min run at 40,000 rpm followed by a 700–1000-min run at 2600 rpm. The slow speeds were found to be ideal for the estimation of diffusion constants from analysis of the boundary spreading. The equations for a homogeneous boundary diffusing in a centrifugal field are represented as follows (Svedberg and Pederson, 1940).

$$D = \frac{\bar{u}^2(1 - s\omega^2 t)}{4y^2 t} \tag{1}$$

$$\frac{c}{c_0} = \frac{1}{2}\left[1 - \frac{2}{\sqrt{\pi}}\int_0^y e^{-y^2}dy\right] \tag{2}$$

In eq 1, $\bar{u}$ is the mean experimental distance in centimeters at a time t from a level in the boundary where the concentration ratio (c/c_0) is 0.5, to two equidistant levels with concentration ratios determined by eq 2. For a definite value of c/c_0, the factor y, the solution of the probability integral $\int_0^y e^{-y^2}dy$, can be found in the Tables of Probability Functions. The influence of a centrifugal field on the diffusion process is given by the factor $(1 - s\omega^2 t)$ in which s is the sedimentation constant under the conditions of the low-speed centrifugation, ω is the angular velocity, and t is the time from the start of the experiment. By combining the intrinsic D so obtained with the intrinsic s deduced from an independent high-speed run, a molecular weight can be calculated from the Svedberg equation.

The sedimentation and diffusion measurements with the ultraviolet system were carried out on samples with an absorbance of 1 OD unit at 260 mμ, so that the resultant sedimentation and diffusion constants correspond to the infinite dilution values, $s^0_{20,w}$ and $D^0_{20,w}$, respectively.

Density measurements for wheat embryo ribosomes were made in 10-ml pycnometers at 20° with samples which had been dialyzed exhaustively against 0.05 M KCl, 0.025 M Tris-HCl (pH 7.5), 0.005 M $MgCl_2$, and 0.006 M β-mercaptoethanol. The ribosome concentrations of these samples were determined spectro-

[1] Abbreviations used: TCA, trichloroacetic acid; T_m, temperature at midpoint of the hyperchromic change; ATP and GTP, adenosine and guanosine triphosphates.

photometrically and were in the range of 0.1–0.6%. The apparent specific volume was calculated from the equation of Schachman (1957) as 0.600 ml/g, in good agreement with the figure of 0.615 ml/g estimated from the assumed partial specific volume ($\bar{v}$) values of the ribosome constituent nucleic acid and protein components by weight, according to the relation

$$\bar{V}_{\text{ribosome}} = (\bar{V}_{\text{RNA}})(w_{\text{RNA}}) + (\bar{V}_{\text{pr}})(w_{\text{pr}}) \qquad (3)$$

where w_{RNA} and w_{pr} are the weight fractions of RNA and protein in the ribosome and $\bar{V}_{\text{RNA}}$ and $\bar{V}_{\text{pr}}$ are assumed partial specific volume values for the nucleic acid and protein constituents (0.53 and 0.72 ml/g, respectively).

Viscosities were measured with Ostwald–Fenske-type viscometers with solvent flow times of ~400–500 sec at 20°. Dust and fibers were removed from the ribosome solutions and solvents by Millipore filtration through 5- and 0.45-μ membranes, respectively, to ensure reproducible flow times.

Optical rotatory dispersion measurements on both wheat embryo ribosomes and RNA preparations were carried out in a Cary 60 recording spectropolarimeter over the wavelength range 550–210 mμ. The slit width of the instrument was programmed to yield maximal and constant light intensity at all wavelengths. The polarimeter tubes used were 1 cm in light path and for measurements in the region of absorption, the absorbance was never allowed to exceed 1.5. The rotations were expressed in terms of specific rotation, $[\alpha] = 100\alpha/cd$, where α is the measured rotation, c is the concentration in g/100 ml, and d is the path length in decimeters.

If it is assumed that the rotations from RNA and protein components within the ribosome are additive, and that the rotations of the isolated RNA in the solution measured are those of the RNA in the ribosome, then protein rotations can be assigned by difference. This subtraction was carried out automatically using the differential cell holder supplied by the Applied Physics Corp. (Palo Alto, Calif.). Ribosomes were placed in one of the 1-cm cells of the assembly and RNA, at the concentration found in the ribosome, was placed in the other matched cell, and a difference spectrum corresponding to the optical rotatory dispersion of the protein was scanned over the wavelength range 550–210 mμ.

The optical rotatory dispersion of the ribosomal protein was determined independently on protein extracted from the ribosomes with cold 66% acetic acid (Waller and Harris, 1961). The resulting acetic acid soluble protein was dialyzed against distilled water for 2 days and then examined in the spectropolarimeter in both the visible and ultraviolet portions of the spectrum. A calculated curve for a hypothetical mixture of 55% rRNA and 45% ribosomal protein, the proportion in which these components occur in the ribosomes, was also determined and compared with the optical rotatory dispersion profile of the intact ribosomes.

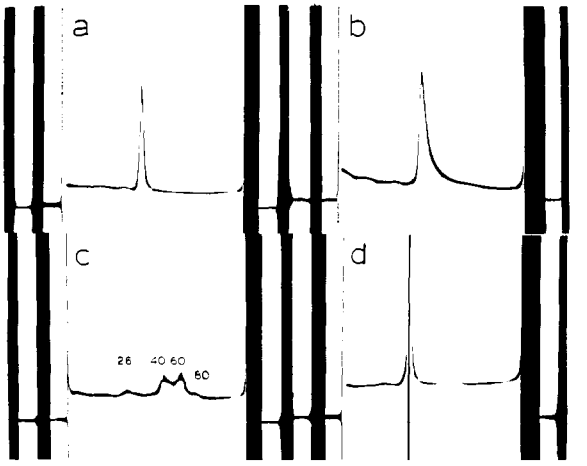

FIGURE 1: Representative sedimentation velocity patterns on (a) native 80S ribosomes in 0.05 M KCl, 0.025 M Tris-HCl (pH 7.5), 0.005 M $MgCl_2$, and 0.006 M β-mercaptoethanol; (b) ribosomes in same buffering medium as a, with elimination of β-mercaptoethanol; (c) ribosomes dialyzed against a medium of 0.05 M KCl, 0.025 M Tris-HCl (pH 7.5), 0.0003 M $MgCl_2$, and 0.006 M β-mercaptoethanol; (d) ribosomes dialyzed against distilled deionized water. Bar angle, 50° throughout; speed of centrifuge 30,000 or 40,000 rpm, direction of sedimentation from left to right, and picture interval from a, b, c, and d as 12, 16, 20, and 12 min, respectively.

Solvent Medium for Physical Measurements. All the hydrodynamic measurements reported herein were carried out in a solvent medium in which the ribosomes were stable and monomeric in the ultracentrifuge; 0.050 M KCl, 0.025 M Tris-HCl (pH 7.5), 0.005 M $MgCl_2$, and 0.006 M β-mercaptoethanol was ideal for this purpose. The polarimeter measurements were effected in the same medium, with the elimination of β-mercaptoethanol which was found to absorb appreciably in the region of the protein conformational Cotton trough. All chemicals used were at least of reagent grade quality.

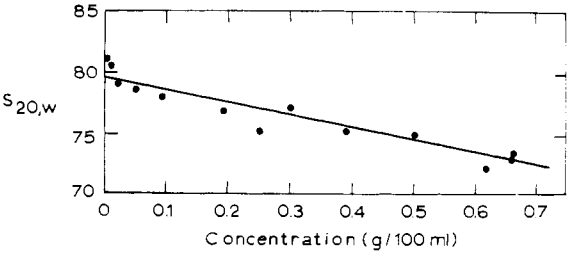

FIGURE 2: Plot of $s_{20,w}$ *vs.* concentration for wheat embryo ribosomes in 0.05 M KCl, 0.025 M Tris-HCl (pH 7.5), 0.005 M $MgCl_2$, and 0.006 M β-mercaptoethanol.

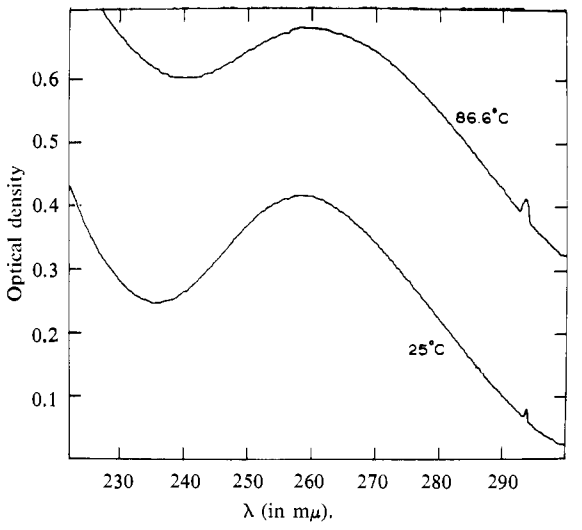

FIGURE 3: Plot of optical density *vs.* wavelength for wheat embryo ribosomes at 25 and 86.6°. (The small peak discernible at ~293 mμ is an instrumental artifact due to a filter cut-in.)

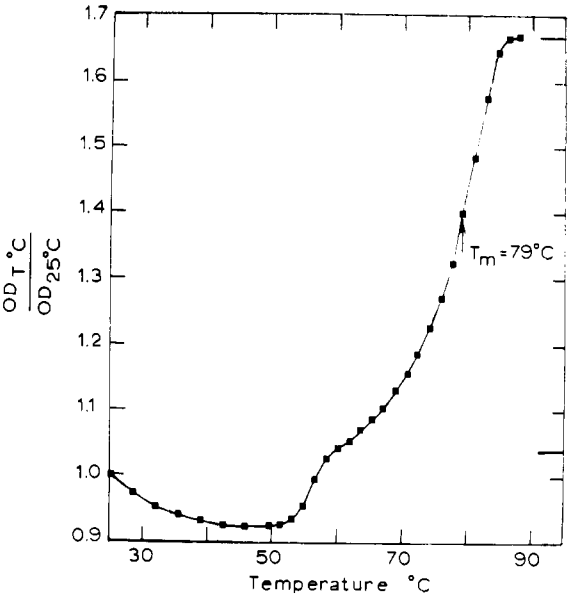

FIGURE 4: Ratio of optical density at $T°$ *vs.* optical density at 25° for wheat embryo ribosomes as a function of temperature.

Results

Sedimentation of Ribosomes. Each ribosome preparation was carefully examined for homogeneity in the ultracentrifuge. A typical schlieren pattern is shown in Figure 1a, which indicates that the ribosomes sediment as a single, homogeneous peak free of lighter and heavier components, and this symmetry is maintained throughout the course of the run. The corrected sedimentation coefficient ($s_{20,w}$) of the ribosomes in the preparative buffering medium was found to be markedly concentration dependent as shown in Figure 2. The intrinsic sedimentation constant ($s^0_{20,w}$), as determined by extrapolation to infinite dilution, is 80 S. This value coincides with that deduced with the ultraviolet absorption system, where the average value of large number of runs on the ribosomes was also 80 S.

The omission of β-mercaptoethanol from the preparative buffer solution results in the association of the ribosomes (Figure 1b); the principal component has an $s^0_{20,w}$ value of 113 S. Tamaoki and Miyazawa (1966) have made a similar observation with *Escherichia coli* ribosomes.

Upon reduction of the magnesium content of the buffer, the ribosomes dissociate reproducibly into discrete subunits. Figure 1c is a typical schlieren sedimentation diagram obtained for a ribosome preparation which was dialyzed for 20 hr against a buffer with only 0.0003 M Mg^{2+} present. A small amount of the 80S component, as well as the 60S, 40S, and 26S subunits are in evidence. (The $s_{20,w}$ values of the subunits were established with the ultraviolet optical system and are therefore the infinite dilution values.) A similar dissociation phenomenon with reduction in magnesium content has been noted by Ts'o *et al.* (1958) for pea seedling ribosomes. The wheat embryo ribosomes behave in a manner similar to the pea seedling system, in that in the absence of magnesium (and at potassium concentrations of 0.05–0.1 M) the ribosomes dissociate completely to the 40S and 26S components (see Table I). Reconstitution of 80S particles by addition of $MgCl_2$ at a final concentration of 0.005 M to a solution containing 40S and 26S subunits was not achievable. At concentrations of 0.001 M magnesium and 0.050 M potassium, the 40S, 60S, and 80S particles are in evidence. It was not possible

TABLE I: The Effect of Varying Magnesium and Potassium Concentrations on the Dissociation of Wheat Embryo Ribosomes.[a]

$MgCl_2$ (M)	KCl (M)	Principal Components ($s^0_{20,w}$ values)
	0.100	26, 40
	0.050	26, 40
	0.010	26, 40, 60
0.0005	0.050	40, 60, 80
0.0010	0.100	26, 40, 60
0.0010	0.050	40, 60, 80
0.0010	0.010	80
0.0050	0.050	80
	(distilled, deionized water)	68

[a] All systems contain 0.025 M Tris-HCl (pH 7.5) and 0.006 M β-mercaptoethanol.

to adjust the Mg^{2+} concentration so that only the 40S and 60S components were present.

Since potassium can replace magnesium within the ribosomal particle, the precise sedimentation pattern obtained will be a sensitive function of the potassium: magnesium ratio in the system. Table I also records that reduction of the KCl concentration in the buffer medium will substantially decrease the extent of the dissociation produced by simultaneously lowering the magnesium concentration, again reminiscent of similar findings noted with pea seedling ribosomes (Ts'o *et al.*, 1958) and reticulocyte ribosomes (Ts'o and Vinograd, 1961). Along these same lines it may be noted that dialysis of the 80S ribosomes against distilled deionized water does not result in the formation of subunits, but rather a homogeneous component with an $s^0_{20,w}$ of 68 S is observed (Figure 1d).

Ultraviolet Absorption Characteristics. Figure 3 portrays the absorption spectrum of the 80S ribosomes at 25° and upon heating to 85°. The absorption maximum at 258 mμ does not change measurably with heating, but the absorption curve itself is uniformly displaced upwards, corresponding to a 70% increase in absorption at 258 mμ. This increase is considerably larger than the 50% increase reported for liver and *E. coli* ribosomes (Hall and Doty, 1959; Zubay and Wilkins, 1960), and may in part be due to the developing turbidity in the solutions as a result of heat coagulation of the ribosomal proteins. The broadening of the absorption maximum at the higher temperature and the decreased depth of the trough at 240 mμ may well be an expression of the same phenomenon, *i.e.*, light scattering by coagulated ribosomal proteins.

The temperature dependence of the absorption maximum at 258 mμ for wheat embryo ribosomes is shown in Figure 4. In order to facilitate comparison of results, the ratio of the optical density at $T°$ *vs.* the optical density at 25° was plotted against increasing temperature. The most striking features of the curve are the initial hypochromism (~10%) upon heating to 50°, the rather abrupt reversion (over a temperature range of 53–60°) of the optical density to its original value at 25°, followed by the marked hyperchromic effect over the temperature range of 60–83°, due largely to the destruction of the secondary structure in the RNA component. The average melting temperature of the ribosomes calculated from the midpoint of the larger hyperchromic change (T_m) is ~79°.

TABLE II: Biological Activity of Wheat Embryo Ribosomes.[a]

Incorporation System	Cpm Recovd
Complete system (15-min incubn)	3200
Complete system (45-min incubn)	8200
Complete system (95-min incubn)	9500
Without ribosomes	390
Without tRNA	240
Without polyuridylate	200
Without supernatant factor	60

[a] The conditions of the incubation and the concentrations of the reagents were as described in Materials and Methods. The incorporating system employed ~250,000 cpm of ^{14}C-labeled phenylalanine as determined by counting the exact amount of pure label added. This value was then corrected automatically for counting efficiency and quenching. All results are reported on 18-hr-old preparations.

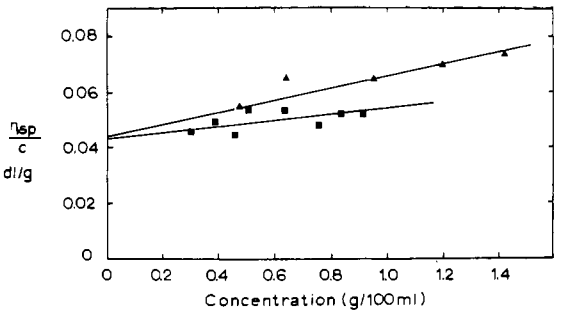

FIGURE 5: Plot of reduced specific viscosity *vs.* concentration for wheat embryo ribosomes in 0.05 M KCl, 0.025 M Tris-HCl (pH 7.5), 0.005 M $MgCl_2$, and 0.006 M β-mercaptoethanol (▲——▲). The solid squares (■——■) pertain to the same solvent system except that the β-mercaptoethanol has been reduced to 0.0006 M.

Assay for Biological Activity. The results of activity studies conducted 18 hr after ribosome preparation are summarized in Table II. The data confirm the earlier findings of Marcus and Feeley (1964) and Allende and Bravo (1966) that the wheat embryo system is virtually free of endogenous phenylalanine mRNA, tRNA, or supernatant factor enzymes. However phenylalanine incorporation can be stimulated more than 40-fold by the addition of polyuridylic acid.

It should be noted that freezing and storage of the ribosomes for 24 hr at 20° followed by thawing results in their precipitation and a complete loss of biological activity. However, they can be stored for longer periods of time in solutions of about 0.5% at 0°. Initially the ribosomes show about 4300-cpm incorporation of [^{14}C]phenylalanine when examined immediately after preparation. At 18 hr after preparation the activity rises to a maximum counts per minute of ~8200, and after 40-hr storage at 0°, the activity falls to ~4300 cpm. Chen *et al.* (1966) reported that pretreatment of wheat embryo ribosomes with trypsin enhanced the synthetic ability when directed by polyuridylate. This finding, coupled with the above time-dependent initial increase and then decrease in activity, may be related to a dissociation of an inhibitory protein from the ribosomes.

Viscosity. The reduced specific viscosity *vs.* the concentration of the wheat embryo ribosomes is presented in Figure 5. The upper curve represents

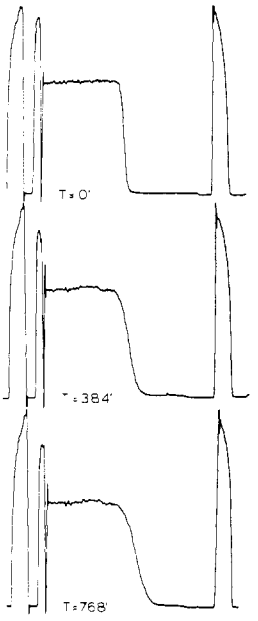

FIGURE 6: Representative ultraviolet absorption patterns of wheat embryo ribosomes during a diffusion run at 2600 rpm. Analytrol tracings correspond to the boundary spreading at different time intervals after deceleration to the low speed.

measurements carried out in the preparative buffer medium of 0.050 M KCl, 0.025 M Tris-HCl (pH 7.5), 0.005 M $MgCl_2$, and 0.006 M β-mercaptoethanol, while the lower curve refers to the identical medium with the exception that the β-mercaptoethanol concentration was reduced to 0.0006 M. It is to be noted that while both plots, by least-squares analysis, extrapolate to a common weight intrinsic viscosity, $[\eta]$, of 0.046 dl/g, their slope terms differ somewhat. Whether this is an inherent artifact or expresses a variable interparticle interaction differing from preparation to preparation is difficult to say.

Diffusion Coefficient Measurements. Figure 6 depicts densitometer traces of boundary patterns corresponding to some of the low-speed pictures of a typical Möller (1964) diffusion run on the wheat embryo ribosomes. Diffusion coefficients were calculated from the spreading of the boundaries using eq 1 and 2 above. Corresponding values of $\bar{u}$ lying on opposite sides of the centroid were determined directly from these analytrol traces. This was achieved by initially drawing two horizontal lines through the plateau areas adjacent to the boundary. From the measured vertical distance between these lines, the 50, 20 (80), and 30 (70)% points were estimated. A representative plot of $(\bar{u})^2(1 - s\omega^2 t)/4y^2$ *vs.* time is presented in Figure 7 for c/c_0 ratios of 20 (80) and 30 (70)% for the ribosomes. That the points derived from the two different sets of pairs of $\bar{u}$ in the boundary fit the same linear curve is indicative of both boundary stability and homogeneity. From the slope of this plot, a $D^0_{3,w}$ value of

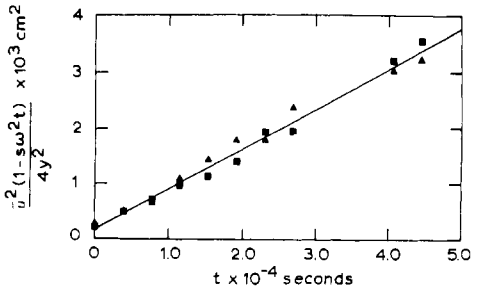

FIGURE 7: A representative plot of $\bar{u}^2(1 - s\omega^2 t)/4y^2$ *vs.* time for wheat embryo ribosomes. The solid triangles (▲——▲) correspond to the 30 (70)% points, while the solid squares (■——■) pertain to the 20 (80)% points.

0.710×10^{-7} cm^2/sec was computed. Correction for temperature and solvent viscosity results in a $D^0_{20,w}$ value of 1.22×10^{-7} cm^2/sec. The average of 15 such determinations on different ribosome preparations resulted in a best average $D^0_{20,w}$ value of 1.15 ± 0.15 Fick units. This value includes the 10% built-in error of this method due to the variation in c_0 and the accuracy of measurement of $\bar{u}$.

Optical Rotatory Dispersion. The ultraviolet optical rotatory dispersion of the wheat embryo ribosomes is highly reproducible, and a representative illustration of the data from one experiment is depicted in Figure 8. The prominent Cotton effect is centered at about 270 mμ, the position characteristic of RNA (Samejima and Yang, 1964), but the existence of an additional feature due to the presence of an α-helical protein is evident in the negative limb. Figure 8 shows, in addition, the optical rotatory dispersion of ribosomal RNA and the optical rotatory dispersion of the protein in the ribosome, deduced by experimentally subtracting the optical rotatory dispersion of the RNA component from that of the ribosome. The protein curve shows the minimum of a negative Cotton effect at 233 mμ, the position characteristic of the α-helical polypeptide chain.

In this study it was assumed that the rotations of the RNA and protein components within the ribosome are additive, and that the rotations of isolated RNA in the solution measured are those of the RNA in the ribosome. Consequently, protein rotations can be assigned by difference. These major assumptions have some experimental support in that with comparable ribosome and bacteriophage systems, the hypochromicity of RNA in solution is about the same as that found for RNA in the ribosome or the phage (Schlessinger, 1960; Zubay and Wilkins, 1960; Day, 1966; McPhee and Gratzer, 1966; Sarkar *et al.*, 1967).

Accepting these assumptions and the assigned protein rotations as first approximations, one can compute reduced mean residue rotations for the protein

$$[m']_\lambda = \left(\frac{3}{n^2+2}\right)\left(\frac{\text{MRW}}{100}\right)[\alpha]_\lambda \qquad (4)$$

by using the refractive index dispersion data tabulated by Fasman (1963) and an assumed mean residue weight (MRW) of 115. This procedure gives $[m']_{233} = -4000°$ for the native ribosomal proteins, a value which, when compared with literature values for helical polypeptides (Blout *et al.*, 1962) indicates a helical content in the vicinity of 14%.

In the visible portion of the spectrum, both the ribosomes and the extracted RNA follow a single-term Drude expression, with $\lambda_c = 259$ and 251 mμ, respectively. Subtracting as before to give the contribution of the protein, the latter is also found to follow the Drude equation, with $\lambda_c = 230$ mμ. This dispersion constant value corresponds to 13% helix, in agreement with the ultraviolet measurements.

The optical rotatory dispersion of the extracted protein in distilled water was measured in both the visible and ultraviolet spectral regions (Figure 8) and the resulting helix parameters were $[m']_{233} = -4000°$, $b_0 = 0°$, and $\lambda_c = 233$ mμ. Helical contents of the isolated protein estimated from these parameters are essentially identical, averaging some 10%. This small α-helix content of the isolated protein compares favorably with the results obtained for the protein *in situ*.

A calculated curve for a hypothetical mixture of 55% rRNA and 45% ribosomal protein (Figure 8), the proportion in which these components occur in ribosomes, mimics closely the optical rotary dispersion pattern displayed by the intact ribosomes. These optical rotatory dispersion studies suggest that the conformation of ribosomal proteins inside the ribosomes is very close to that in the free state.

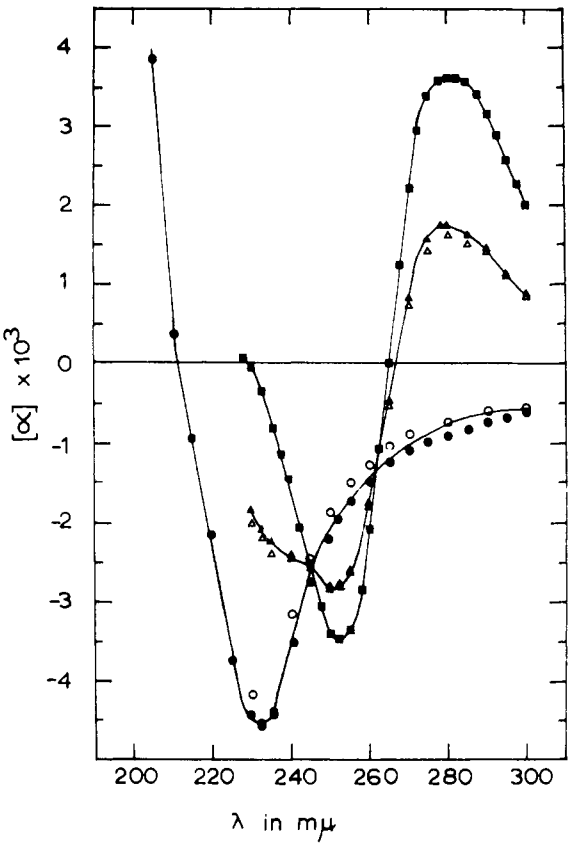

FIGURE 8: Plot of the specific rotation *vs.* wavelength in the ultraviolet region for wheat embryo ribosomes and their constituent RNA and proteins. The solid triangles (▲——▲) correspond to the intact ribosomes, the solid squares (■——■) pertain to the extracted rRNA, and the solid circles (●——●) refer to the extracted protein. The open triangles (△——△) represent the calculated optical rotatory dispersion pattern of the ribosomes, and the open circles (○——○) refer to the calculated protein, obtained by difference spectropolarimetry.

Discussion

It is possible to prepare ultracentrifugally homogeneous and stable 80S ribosomes from wheat embryos. These particles possess a higher percentage of RNA (55%) than that reported for plant ribosomes from other sources (40–45°), but comparable and higher RNA contents have been noted for some microbial and animal ribosomes (see Petermann, 1964). Three ultracentrifugally indentifiable components with intrinsic sedimentation constants of 60, 40, and 26 S, respectively, are obtained as dissociation products when Mg^{2+} is removed from the ribosome. In this respect, wheat embryo ribosomes are similar to those from pea seedlings (Ts'o *et al.*, 1958).

The molecular weight of the 80S ribosomes can be calculated from the sedimentation diffusion data reported in this study by the use of the Svedberg equation (Svedberg and Pedersen, 1940)

$$M = \frac{RTs}{D(1 - \bar{v}\rho)} \qquad (5)$$

where R is the gas constant, T the absolute temperature, ρ the solvent density, $\bar{v}$ the partial specific volume, and s and D the intrinsic sedimentation and diffusion constants, respectively. Substitution of the appropriate experimental values into this relationship results in a calculated molecular weight value of 4.25 (±0.5) × 10^6) for the 80S particles. (Unfortunately it was not possible to determine the diffusion constants of the subunits by the Möller method because of inadequate resolution of the boundaries.)

Preliminary electron microscope observations on wheat embryo 80S ribosomes (kindly carried out by Dr. T. Yamamoto, Department of Microbiology) suggest that the particles are essentially spherical in shape. On the assumption that the ribosomes are spheres, their hydrated diameter, calculated from the experimental diffusion coefficient and the relation $D = RT/6\pi\eta rN$, is 37.0 mμ, which is consistent with a hydrated sphere having 3.17 g of water/g of dry weight of ribosome. The hydrated volume (26.5 × 10^6 A^3) is 5.5 times the dry volume of 4.21 × 10^6 A^3

obtained from $M\bar{V}$, suggesting that the wheat embryo ribosome contains approximately 80% of its enclosed volume as water of hydration. This water of hydration may be internal, in which case the ribosome would be a porous, spongelike structure or alternatively it may reside as an extensive hydration shell about the particle. The former possibility is favored with reticulocyte ribosomes on the basis of low-angle X-ray diffraction studies (Dibble and Dintzis, 1960; Dintzis *et al.*, 1958). If ribosomes are so highly porous, it seems obvious that they cannot be treated hydrodynamically as uniform solid impermeable objects, and calculations of the dimensions of such particles from rigid model forms will not be meaningful.

The biological activity studies reported herein confirm the earlier reports of Marcus and Feeley (1964), Allende and Bravo (1966), and DeGroot *et al.* (1967) that an amino acid incorporating system may be obtained from ungerminated wheat embryo. This system has very low endogenous activity, but phenylalanine incorporation can be stimulated by the addition of polyuridylate. The relative ease of obtaining large quantities of highly pure, synthetically active ribosomes renders this system an ideal one for studying protein synthesis *in vitro*.

X-Ray diffraction studies of *E. coli* ribosomes (Zubay and Wilkins, 1960) and studies on their hypochromicity (Schlessinger, 1960) have indicated that the structure of RNA in the ribosome is essentially the same as in the free state. In the interpretation of our optical rotatory dispersion data we have made the reasonable assumption that the optical rotatory dispersion of the RNA in the ribosome is the same as that of the free molecule in solution. Using this approach, difference spectropolarimetry indicates that the α-helical content of wheat embryo ribosomal proteins is very low ($\sim$10%), as estimated from b_0 measurements in the visible region and $[m']_{233}$ calculations in the ultraviolet region. A similar conclusion was deduced from comparable measurements on extracted ribosomal proteins, which would suggest that the conformation of proteins inside the ribosome is also very close to that in the free state. This premise is further substantiated by the optical rotatory dispersion summation experiment in which the pattern generated from a hypothetical mixture of 55% RNA and 45% protein closely resembled the optical rotatory dispersion pattern displayed by the intact ribosome. The small α-helical content of ribosomal proteins is not unexpected, in view of their similar compositional makeup to the small, highly basic protamines which possess little α helix (Yang and Doty, 1957).

Our optical rotatory dispersion results on the wheat embryo system are very similar to those discerned for the ribosomes of rabbit reticulocytes (Blake and Peacocke, 1965), yeast (McPhee and Gratzer, 1966), and *E. coli* (Sarkar *et al.*, 1967). These studies as well as our own suggest that the protein must be associated with the RNA in the ribosome in such a manner as to have little or no influence on the conformation of either constituent alone, and in this way there is a minimum coupling of rotatory effects.

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Discovery of Ubiquinones-1, -2, -3, and -4 and the Nature of Biosynthetic Isoprenylation*

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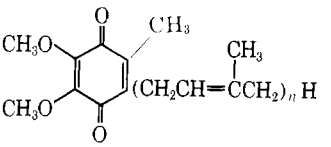
ABSTRACT: High-sensitivity mass spectrometry of paper chromatographically pure samples of the dominant ubiquinone from three diverse microorganisms has revealed the presence of trace amounts of ubiquinones-1, -2, -3, and -4. The presence of these ubiquinones in bacteria was confirmed by special chromatographic techniques using paraffin-impregnated thin layer plates. Ubiquinones-1, -2, -3, and -4 have previously been known by synthesis, but not by natural occurrence. Recently, 2-tetraprenylphenol, which is a precursor of ubiquinone-4, has been isolated from *Rhodospirillum rubrum*, and ubiquinone-5 has been isolated from *Escherichia coli*. The entire series, ubiquinones-1 through -10, is now known from nature. Pure ubiquinone-6 from *Saccharomyces cerevisiae* revealed the presence of ubiquinones-1 through -6. Similarly, pure ubiquinone-8 from *E. coli* revealed the presence of ubiquinones-1 through -8, and pure ubiquinone-10 from *R. rubrum* revealed ubiquinones-1 through -10. The difference in the concentrations of the ubiquinones at the extremes in molecular weight could be 500- to 1000-fold. There is no absolute structural specificity in certain species for the biosynthetic formation of a single multiprenyl pyrophosphate or in its reaction with *p*-hydroxybenzoic acid. Instead, biosynthetic isoprenylation takes place so that a series of ubiquinones are formed in which the number of isoprene units contained in the side chains varies from the number of units in the dominant ubiquinone for a given species (6, 8, or 10 for the species studied) to one. The relative concentrations of the ubiquinones diminish with diminishing chain length.

The occurrence in bacteria of ubiquinones-1, -2, -3, and -4 (Q_{1-4}, I, n = 1, 2, 3, and 4, respectively)[1] has been discovered. The existence in nature of ubiquinones-6 through -10 has been recognized (Lester and Crane, 1959) for a number of years. Recently, the isolation of a pure sample of Q_5 (I, n = 5) from *Escherichia coli* was reported (Friis *et al.*, 1966a), and 2-tetraprenylphenol, a precursor of ubiquinone-4, was characterized from *Rhodospirillum rubrum* (Olsen *et al.*, 1966). With the detection of ubiquinones-1 through -4 in nature, all of the ubiquinones with side chains of from one to ten isoprene units are now recognized as constituents of living systems, although these new lower isoprenylogs occur in very minor amounts as compared to the corresponding major ubiquinone of a given species.



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Mass Spectrometric Studies. The initial examination by high-sensitivity mass spectrometry of a paper chromatographically pure sample of Q_6 isolated from *Saccharomyces cerevisiae* revealed the presence of peaks which correspond to lower molecular weight ubiquinones. In addition to the peaks expected for Q_6 (Muraca *et al.*, 1967), peaks were observed (Figure 1a, top) which could be assigned to parent ions (M) of Q_5, Q_4, Q_3, Q_2, and Q_1. In each case, peaks of appropriate relative intensities due to the molecular ions of the corresponding hydroquinones were also observed. The observation of peaks due to hydroquinones is in accord with earlier data on the mass spectra of the ubiquinones (Muraca *et al.*, 1967) and the plastoquinones (Das *et al.*, 1965) in which peaks at M + 2 due

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[1] Abbreviations used: Q_{1-4}, ubiquinones-1, -2, -3, and -4; HBA, *p*-hydroxybenzoic acid.