PHYSICAL CHARACTERISTICS OF THE RECONSTITUTION INTERMEDIATE $RI_{50(1)}$ FROM THE 50 S RIBOSOMAL SUBUNIT OF *ESCHERICHIA COLI*

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1. Introduction

The procedure for the in vitro reconstitution of active 50 S ribosomal subunits from *Escherichia coli* first appeared in 1974 [1]. The same group later reported the kinetic study of the reconstitution steps and gave the estimated sedimentation values of the reconstitution intermediates in sucrose gradient in 1976 [2]. Due to the relative difficulty in isolating the intermediates, no physical studies of these intermediates have yet been reported. We have isolated the first intermediate $RI_{50(1)}$, which is homogeneous and stable under low salt conditions. We report here the physical characteristics of the $RI_{50(1)}$ particle as determined by hydrodynamic methods and compare our results with the intact 50 S subunits, which have been characterized under the same buffer conditions.

2. Materials and methods

2.1. *Preparation of 50 S subunits*

Ribosomal subunits were prepared using the methods in [3]. The 50 S and 30 S subunits were separated on a 10–30% exponential sucrose gradient formed in a Ti-15 Beckman zonal rotor spun at 32 000 rev./min for 14.5 h. The 50 S subunits were recovered from the sucrose fractions by precipitating with 2 vol. ethanol after raising Mg^{2+} and dithiothreitol to 0.01 M and 0.001 M, respectively. The precipitate was pelleted by centrifugation at 20 000 × *g* for 30 min, dissolved and dialyzed at 4°C against 0.01 M Tris–HCl (pH 7.4), 0.1 M KCl and 1.5 mM $MgCl_2$ (buffer 1) overnight. The purity of the ribosomal preparation was checked routinely by means of sedimentation velocity in the Beckman model E analytical ultracentrifuge.

2.2. *Preparation of $RI_{50(1)}$*

Total proteins from the 50 S subunits (TP 50) and the 23 S and 5 S RNA were prepared as in [2]. For each A_{260} unit of 23 S and 5 S RNA, 2 equivalent units of proteins were added. The mixture was incubated at 0°C for 30 min, and immediately placed on a 10–36% exponential sucrose gradient prepared with the reconstitution mixture (0.02 M Tris–HCl (pH 7.6), 0.004 M $Mg(OAc)_2$, 0.4 M NH_4Cl, 0.0002 M EDTA and 0.002 M β-mercaptoethanol). The sample was centrifuged in a Ti-14 Beckman zonal rotor spun at 48 000 rev./min for 8 h. The $RI_{50(1)}$ particles were recovered from the sucrose solution by precipitation with 2 vol. cold 95% ethanol. The precipitate was collected by centrifugation, dissolved in buffer 1, and dialyzed against the same buffer for ⩾36 h with 3 changes of dialyzate before physical measurements were made.

2.3. *Physical measurements*

The apparent sedimentation coefficient of the 50 S subunit and $RI_{50(1)}$ particles were determined using a Beckman model E ultracentrifuge at 48 000 rev./min at 4°C with an ANE rotor. This value was corrected for temperature and solvent differences to give $s_{20,w}$. These values obtained at varying concentrations were then extrapolated to infinite dilution to give $s^{\circ}_{20,w}$.

The density increment $(\partial\rho/\partial c)$ [4] was obtained from a density *vs* concentration plot. The density of each sample was determined using Paar DMA-02C digital density meter following the procedure in [5]. The concentration of each sample was determined spectrophotometrically using an extinction coefficient of $E^{1\%}_{260} = 152$. The slope of the density *vs* concentration plot $(\partial\rho/\partial c)$ was determined by a linear least-squares program. The apparent specific volume for the parti-

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cle was obtained by using the equation:

$$\Phi^* = \frac{1}{\rho_o}\left(1 - \frac{\partial\rho}{\partial c}\right)$$

where ρ_o is the density of the dialysate and Φ^* is the apparent specific volume.

The extinction coefficient was determined by measuring the A_{260} of solutions, then determining the concentration of those solutions by means of dry weight measurements [6].

Diffusion coefficients were determined using intensity fluctuation spectroscopy [7–9] from the correlation function:

$$g(\tau) = e^{-DK^2\tau}$$

where $g(\tau)$ is the normalized first order electric field correlation function, τ is the delay time, K is the magnitude of the scattering vector and D is the translational diffusion coefficient. The diffusion measurements were made directly on the sample in cuvette, using a Malvern 4300 spectrometer system containing a digital autocorrelator.

The molecular weight (M) was determined by combining the diffusion coefficient, sedimentation coefficient, and the density increment ($\partial\rho/\partial c$) in the Svedberg equation:

$$M = \frac{sRT}{D\left(\frac{\partial\rho}{\partial c}\right)}$$

where R is the gas constant and T is the absolute temperature.

The frictional coefficient ratio f/f_{min} [10] is obtained by using:

$$f/f_{min} = \frac{(4/3)^{1/3}}{6\eta(\pi N)^{2/3}} \cdot \frac{(1 - \Phi^*\rho_o)M^{2/3}}{(\Phi^*)^{1/3}s}$$

where ρ_o, η are density and viscosity of the solvent, respectively, and N is Avagadro's number.

The effective hydrodynamic radii [10] for the $RI_{50(1)}$ and 50 S can be calculated by using the equation:

$$R_{sed} = \frac{M(1 - \Phi^*\rho_o)}{6\pi\eta Ns}$$

where the symbols are the same as defined above.

2.4. *Protein composition*

The protein composition of the $RI_{50(1)}$ particle was established by two-dimensional gel electrophoresis [11] after extraction with 67% acetic acid in the presence of 0.1 M $MgCl_2$ [12].

3. Results and discussion

3.1. *Protein composition*

The isolated $RI_{50(1)}$ particle contains 21 proteins as listed in table 1. These results indicate that the $RI_{50(1)}$ particle contains fewer proteins than reported

Table 1
Protein composition of $RI_{50(1)}$ particle

	This study	Nierhaus et al. [13]
L1	+	+
L2	+	+
L3	+	+
L4	+	+
L5	(±)	+
L6	–	–
L7	+	–
L8	+	–
L9	+	+
L10	+	+
L11	+	+
L12	+	–
L13	+	+
L14	–	–
L15	+	–
L16	–	–
L17	+	+
L18	–	–
L19	(±)	+
L20	+	+
L21	+	+
L22	+	+
L23	+	+
L24	+	+
L25	–	–
L26	–	–
L27	–	–
L28	–	–
L29	(±)	+
L30	–	–
L31	–	–
L32	–	–
L33	–	+
L34	–	–

Symbols: +, present in normal amount; (±), present in trace amount; –, absent

in [2], but in close agreement with the data in [13]. There are 4 proteins (L7, L8, L12, L15) which we found on the $RI_{50(1)}$ particle that have not yet been reported. Protein L33 was reported to be on the $RI_{50(1)}$ [2,13] but was not found in our intermediate. In [13] the proteins on 50 S subunit were classified into 'late assembly proteins' and 'early assembly proteins', according to their order of assembly during the reconstitution process. All the proteins assigned as 'early assembly proteins' [13] are present on the $RI_{50(1)}$ particle we isolated. Proteins L7, L8 and L12 were classified as 'unassigned proteins' but were found in normal amounts on our particle.

If we add the molecular weights of the attached proteins to the molecular weight of the 23 S and 5 S RNAs (1.1×10^6), we obtain mol. wt $\sim 1.49 \times 10^6$ for the reconstitution intermediate.

3.2. *Physical measurements*

The physical characteristics for the $RI_{50(1)}$ particle are listed in table 2 together with the values for 50 S subunit in the same buffer for comparison. All of the data indicate that the reconstitution intermediate is highly unfolded. The $s^\circ_{20,w}$ value of $32.4 \pm .4$ is essentially the same number as that obtained by sucrose gradient measurements. If the intermediate were to have essentially the same conformation as the 50 S particle, it would be expected to have a sedimentation value of ~48 S. The diffusion coefficient of $1.23 \pm 0.02 \times 10^{-7}$ cm^2/s is substantially less than that of the 50 S subunit ($1.9 \pm .04 \times 10^{-7}$ cm^2/s), and indicates an extended conformation as well. We obtained $E^{1\%}_{260} = 152$, which is slightly higher than the 50 S subunit which may indicate a more extended conformation. The partial specific volume and density increment for the particle are 0.568 ml/g and 0.429, respectively. By combining the S, D and $(\partial\rho/\partial c)$ terms in the Svedberg equation, we obtained mol. wt = 1.49×10^6, which is the value estimated from protein composition calculation. The calculated f/f_{min} and effective hydrodynamic radii R_{sed} are 2.43 and 167 Å for the reconstitution intermediate and 1.63 and 112 Å for the 50 S subunit, respectively.

Table 2
Physical characteristics of $RI_{50(1)}$ particle and 50 S subunit in buffer 1

	$RI_{50(1)}$	50 S
$s^\circ_{20,w}$ (S)	32.4 ± 0.4	50.2 ± 0.5
$D^\circ_{20,w}$ (cm^2/s × 10^{-7})	1.23 ± 0.02	1.90 ± 0.04
$E^{1\%}_{260}$	152	145
Φ^* (g/ml)	0.568 ± 0.005	0.592 ± 0.006
$\frac{\partial\rho}{\partial c}$	0.429	0.412
f/f_{min}	2.43	1.63
R_{sed} (Å)	167	112

From all of these physical data, we can conclude that the reconstitution intermediate has an unfolded conformation and that the attachment of the 21 proteins onto the RNA does not cause the intermediate to assume a 50 S subunit conformation at this stage of reconstitution.

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