PHYSICAL CHARACTERISTICS OF 23 S rRNA FROM THE 50 S RIBOSOMAL SUBUNIT OF *ESCHERICHIA COLI*

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1. Introduction

The in vitro reconstitution of the bacterial 30 S ribosomal subunits [1–2] and subsequently the 50 S subunits [3–5], has established the fact that the information required for the assembly of the subunits is present in the structure of the proteins and/or RNAs. Previous work has also shown that the reconstitution process requires a rigid set of buffer conditions.

Recently, Vasiliev and Zalite [6], using electron microscopy, concluded that the 23 S rRNA molecules are capable of acquiring a specific compact conformation which is morphologically close to intact 50 S subunits. However, their RNA was prepared in the presence of EtOH and spermidine, which have been shown to cause conformational changes on bacterial ribosomes [7,8]. Sieber et al. [9] also reported an electron microscopic analysis of the 23 S rRNA, which has an overall diameter larger than the dimensions reported by Vasiliev and Zalite [6], as well as the 50 S subunit [10]. From these disparate results it is apparent that further physical studies are needed to clarify the structure of 23 S rRNA.

We report here the physical characteristics of the 23 S rRNA under buffer conditions used for the formation of the first reconstitution intermediate, as well as under low salt conditions normally used with hydrodynamic methods. We have compared these results with the hydrodynamic properties obtained previously on the first reconstitution intermediate $RI_{50(1)}$ [11].

Our results indicate that under reconstitution conditions, the conformation of the 23 S rRNA is considerably more extended than that found in the 50 S subunit, and that upon addition of protein the RNA structure does not appear to be appreciably affected.

2. Materials and methods

2.1. *Preparation of 50 S subunits*

Ribosomal subunits were prepared using previously described methods [12]. 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 volumes of ethanol after raising the Mg^{2+} and dithiothreitol concentration to 0.01 M and 0.001 M. 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 7.6, 0.01 M $Mg(OAC)_2$, 0.06 M NH_4Cl and 0.004 M β-mercaptoethanol overnight. The purity of the ribosomal preparations was checked routinely by means of sedimentation velocity in the Beckman Model E analytical ultracentrifuge.

2.2. *Preparation of 23 S rRNA*

The 23 S and 5 S rRNAs were extracted from the 50 S subunits with phenol according to the method of Nierhaus et al. [5]. After the final extraction, the rRNA samples were precipitated with 2 volumes of EtOH, 20 mM Tris–HCl, pH 7.8, and pelleted at 10 000 rev./min for 30 min at 4°C in an HB-4 rotor. The pellets were dissolved in and dialyzed against 0.01 M Tris–HCl, pH 7.4, 0.1 M KCl and 0.0015 M $MgCl_2$ (Buffer I). The 23 S rRNA was separated from the 5 S rRNA by passing through a 10–30% exponential sucrose gradient formed in a Ti-14 Beckman zonal rotor spun at 48 000 rev./min for 9.5 h. The 23 S rRNA was precipitated with 95% EtOH and collected by centrifugation. The RNA was then dissolved in and dialyzed against either Buffer I or Recon Buffer (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) to equilibrium with three changes of dialyzate before physical measurements were made.

2.3. *Physical measurements*

The apparent sedimentation coefficients of the 23 S rRNA at various concentrations in either Buffer I or Recon Buffer were determined using a Beckman Model E Ultracentrifuge at 48 000 rev./min at 20°C with an ANE rotor. These values were corrected for solvent differences to give $s_{20,w}$ and then extrapolated to infinite dilution to give $s^{\circ}_{20,w}$.

The density increment $(\partial\rho/\partial c)$ [13] was obtained from a density vs. concentration plot. The density of each sample was determined at 20°C using a Paar DMA 02C digital density meter following the procedure previously outlined [14]. The concentration of each sample was determined spectrophotometrically using an extinction coefficient of $E^{1\%}_{260} = 201$. 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 particle was obtained by using the equation:

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

where $\rho_\circ$ is the density of the dialysate and Φ^* is the apparent specific volume.

The extinction coefficient was determined by measuring the absorbance of solutions at 260 nm and then determining the concentration of those solutions by means of dry weight measurements [15].

Diffusion coefficients were determined using intensity fluctuation spectroscopy [16–18] 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. Samples for diffusion measurements were banded in a 10–30% sucrose gradient using a Beckman SW 50.1 rotor and run at 45 000 rev./min for 3.5 h at 20°C. The diffusion measurements were made directly on the sample banded in the centrifuge tube, using a Malvern 4300 Spectrometer system containing a digital autocorrelator.

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

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

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

The frictional coefficient ratio f/f_{min} [19] is obtained by using the equation:

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

where $\rho_\circ$ and N are the density and viscosity of the solvent respectively and N is Avogadro's number.

The effective hydrodynamic radii [19] for the 23 S rRNA can be calculated by using the equation:

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

where the symbols are the same as defined above.

3. Results and discussion

The physical characteristics for the 23 S rRNA in Recon Buffer and Buffer I are listed in table 1 together with the values for $RI_{50(1)}$ and 50 S subunit in Buffer I [9] for comparison.

In Recon Buffer, the 23 S rRNA has a sedimentation coefficient $s^{\circ}_{20,w}$ of 26.3 ± 0.3 S and a diffusion coefficient $D^{\circ}_{20,w}$ of 1.34 ± 0.04 × 10^{-7} cm^2 s^{-1}. The 23 S rRNA in Buffer I has a sedimentation coefficient $s^{\circ}_{20,w}$ of 25.7 ± 0.4 S and a diffusion coefficient $D^{\circ}_{20,w}$ of 1.33 ± 0.02 × 10^{-7} cm^2 s^{-1}, which are identical to the values obtained in Recon Buffer.

From these results it appears that the frictional coefficient of the RNA is identical in the two different buffer systems, which in turn implies that the gross conformation of the RNA is identical in both buffer systems. This is also reflected in the values of the hydrodynamic radii, which are identical.

The density increments $(\partial\rho/\partial c)$ for the RNA in Recon Buffer and Buffer I are 0.404 and 0.462. The corresponding apparent specific volumes are 0.593 ± 0.006 ml g^{-1} for the RNA in Recon Buffer,

Table 1
Physical characteristics of 23 S rRNA, $RI_{50(1)}$ particle and 50 S subunit

	Recon Buffer	Buffer I		
	23 S rRNA	23 S rRNA	$RI_{50(1)}$[a]	50 S[a]
$s^{\circ}_{20,w}$	26.3 ± 0.3	25.7 ± 0.4	32.4 ± 0.4	50.2 ± 0.5
$D^{\circ}_{20,w}$ ($cm^2\ s^{-1} \times 10^{-7}$)	1.34 ± 0.04	1.33 ± 0.02	1.23 ± 0.02	1.90 ± 0.04
$E^{1\%}_{260}$	201	201	152	145
Φ^* (ml g^{-1})	0.593 ± 0.006	0.536 ± 0.006	0.568 ± 0.005	0.592 ± 0.006
$\partial\rho/\partial c$	0.404	0.462	0.429	0.412
f/f_{min}	2.44	2.68	2.43	1.63
R_{sed} (Å)	159	161	167	112
Mol. wt × 10^6	1.18 ± 0.06	1.02 ± 0.05	1.49 ± 0.07	1.56 ± 0.06

[a] Data from [11]

and 0.536 ± 0.006 ml g^{-1} for the RNA in Buffer I. The higher apparent specific volume for the 23 S rRNA in Recon Buffer may be explained by the presence of high concentration of ammonium ions. Since the partial specific volume for ammonium ion (+1.0 ml g^{-1}) is more than four times the value for potassium ion (+0.225 ml g^{-1}) [20,21], replacing the potassium ion with the ammonium ion may possibly result in an increase in the apparent partial specific volume of the salt-associated RNA.

In any case it is apparent that there is a considerable difference in the apparent specific volumes which, if the gross conformations of the RNA in the two buffer systems are really identical, implies that there is a substantial change in the solvent-biopolymer interaction. This is corroborated by the differences in molecular weights and frictional coefficient ratios (f/f_{min}) of the RNA in the two different buffer systems.

The molecular weight results are obtained by combining the s, D and $(\partial\rho/\partial c)$ terms in the Svedberg equation. We obtained molecular weights of 1.18×10^6 and 1.02×10^6 for the RNA in Recon Buffer and Buffer I respectively. The calculated molecular weights are higher than the estimated value from sequencing (0.94×10^6 [22]) and can be attributed to varying salt association effects in the two buffer systems.

The extinction coefficients for the RNA in both buffers as determined by dry weight are identical and have a value of $E^{1\%}_{260}$ of 201 ± 4. The extinction coefficient we obtained is lower than the corresponding value ($E^{1\%}_{260}$ = 223) for the 23 S rRNA in a buffer that contains only 0.05 M Tris–HCl, pH 7.4, 0.1 M KCl (Ortega, J. P., unpublished results). This difference is probably due to the presence of Mg^{2+} in Buffer I and Recon Buffer, which enhances base pairing and base stacking, resulting in a concommitant decrease in the absorptivity of the sample.

Comparison of the 23 S rRNA with the $RI_{50(1)}$ particles is more difficult due to a lack of knowledge of the hydration of the RNA and the ribonucleoprotein complex. The $RI_{50(1)}$ particle has a molecular weight about 33% greater than that of the 23 S rRNA. The sedimentation coefficient of the $RI_{50(1)}$ particle should be approximately 34.4 S if the proteins were added in such a way as to cause no change in the asymmetry or hydration of the particle. Instead we reported an $s^{\circ}_{20,w}$ of 32.5 S for the $RI_{50(1)}$ particles. This particle also has a lower diffusion coefficient and a slightly higher R_{sed} than the RNA. Apparently there is a slight increase in the axial asymmetry and/or hydration in the resulting ribonucleoprotein structure upon the binding of the protein suggesting that in forming the $RI_{50(1)}$ particle, protein binding does not cause substantial folding or tightening of the RNA.

The physical characteristics of the 50 S subunit are drastically different from either those of the RNA and/or the $RI_{50(1)}$ particle. The changes cannot solely be attributed to a decrease/increase in hydration. The conformation of the RNA inside the 50 S subunit is definitely more compact than either the free RNA or that of the RNA inside the $RI_{50(1)}$ particle.

These results are at variance with those found by

Vasiliev et al. [6] who used electron microscopy and concluded that the 23 S rRNA can exist in a compact form which is morphologically close to the structure of intact 50 S subunits, even though the reported length for the RNA (260 ± 20 Å) is slightly larger than the 230 ± 10 Å seen for the 50 S subunit. However, the RNA preparations studied were prepared in buffers containing 1 M EtOH and 2 mM spermidine. Although the effect of EtOH on RNA preparations has not been thoroughly documented to our knowledge, Bernabeu et al. [8] have suggested that MeOH changes the conformation of rRNA by affecting the hydrophobic interactions necessary for the tertiary structure of the rRNA. Similar effects with EtOH should be expected. The elaborate studies of Weiss and Morris [23] on the 30 S subunit, and Kimes and Morris [7] on the 50 S subunit have established the effect of spermidine on the conformation and activity of the ribosomal subunits. Undoubtedly, the difference between our results and those of Vasiliev et al. can be explained by the difference in buffer conditions.

Recent studies by Sieber et al. [9] corroborate the results we found hydrodynamically. They studied 23 S rRNA preparations in reconstitution conditions using electron microscopy and concluded that the rRNA has an overall diameter of 310–330 Å which is considerably larger than that of the 50 S subunit (170 × 110 Å) under the same conditions.

It is very apparent that the buffer and salt conditions affect the structure of the RNA enormously. Due to ionic strength and salts present the base stacking, base pairing and electrostatic interactions can be strongly affected causing the observed changes in tertiary structure. Only by studying the RNA under conditions of reconstitution and activity can relevant structures be obtained.

In summary, we conclude that the 23 S rRNA in either Recon Buffer or low salt buffer (Buffer I) has a more unfolded or extended conformation than the 50 S subunit. The conformation of the free 23 S rRNA cannot fit into the dimensions of the intact 50 S subunit.

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