Structure and Stability of the Reconstituted Intermediate Particles Involved in the in Vitro Self-Assembly of the 30S Ribosomal Subunit†

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ABSTRACT: In an attempt to decipher further the molecular mechanism of the self-assembly of the 30S ribosomal subunit, we have studied the hydrodynamic properties, conformation, and stability of the major reconstitution intermediate, RI, and its heat-activated form, RI*. Preliminary hydrodynamic parameters (f/f_{min} or R_{eff}) of RI are similar to those of the 30S particle, while those for RI* are similar to those of the 16S RNA. This indicates that, during the course of the 30S assembly, the hydrodynamic shape and/or compactness of 16S RNA appear to switch back and forth as the 16S RNA proceeds through the RI, then the RI*, and finally the 30S subunit. The melting of the 16S RNA, RI*, and 30S subunit was studied simultaneously by three different conformational parameters, and all show complex behavior through the whole temperature range studied (25–90 °C). The RI* is similar to the 16S RNA at low temperature (<50 °C) and closer to the 30S subunit at high temperatures (>80 °C), and its behavior is intermediate between that of the 16S RNA and 30S subunit in the middle of the transition (50–80 °C). The bound proteins in the RI* and the 30S subunit appear to stabilize the RNA against melting. The conformational changes of the proteins and the RNA during the heat activation of RI to RI* and the cooling of RI* to RI* at 5 °C have been further investigated by studies of the component RNA and the proteins alone. The conformational changes of the free proteins and RNA upon heating are similar to those observed for the heat activation of RI to RI*. However, complete reversibility was observed for the free proteins and RNA alone, contrary to the complete irreversibility of the proteins and ~75% reversibility of the RNA in the RI* for the heat activation of RI to RI*. The results suggest that the conformational changes are initiated by the 16S RNA and the proteins alone, but the protein–RNA interactions in the RI and RI* particles results in irreversible conformational changes of the proteins upon cooling.

The extensive studies of Nomura and co-workers [e.g., Traub & Nomura (1969), Held & Nomura (1973), and Nomura (1973)] show that the in vitro assembly of the *Escherichia coli* 30S ribosomal subunit follows the sequential steps

16S RNA + RI proteins (S4-S9, S11-S13, S15-S20) $\xrightarrow{0\text{–}10\ °C}$ reconstitution intermediate (RI) (1)

RI $\xrightarrow{37\ °C}$ activated reconstitution intermediate (RI*) (2)

RI* + split proteins ([S1], S2, S3, S10, S14, [S21]) $\xrightarrow{0\text{–}37\ °C}$ 30S subunit (3)

At 10 °C or lower, the inactive RI[1] particle, which contains 16S RNA and 15 of the 30S ribosomal proteins, is assembled. This is the stable intermediate which undergoes the major rate-limiting step that requires the high activation energy and represents a unimolecular structural rearrangement which follows first-order kinetics (Traub & Nomura, 1969). Isolated RI can be heated to 37 °C in the absence of free split proteins to form an RI* particle which can bind the split proteins to form the 30S subunit. The RI* can be cooled to 5 °C and retains its split protein binding activity to form the 30S subunit. Mangiarotti et al. (1974) have found that 30S precursors isolated from whole cells in vivo will form ribosomes when incubated with ribosomal proteins at 0 °C. At present, the rate-limiting step of in vitro 30S assembly appears to be very similar to that which occurs in vivo. Reconstitution, genetic, and physiological studies all tend to indicate the presence of a strong, major kinetic holdup point that is probably universal.

Despite the extensive elegant studies on the assembly of 30S ribosomal subunits, and more recently of the *E. coli* 50S subunit [e.g., Spillmann et al. (1977)], much about the *molecular mechanisms* which provide for the recognition and interaction of the molecular component proteins and RNA remains to be elucidated. Several investigators have suggested that the in vivo 30S assembly involves a rearrangement of the 16S RNA molecule [e.g., Nashimoto & Nomura (1970) and Wireman & Sypherd (1975)]. Held & Nomura (1973) reported that RI and RI* differ in their sedimentation coefficients. Hochkeppel & Craven (1977) observed electrophoretic differences in the 16S RNA isolated from RI and RI* particles. The conformational difference of proteins free in solution and that of proteins bound in the ribosomal particle have also been investigated with conflicting results (McPhie & Gratzer, 1966; Sarkar et al., 1967). A variety of other approaches have also been attempted to decipher the molecular bases of the assembly (Wong & Dunn, 1974; Wong et al., 1974; Bruce et al., 1977; Fox & Wong, 1978; Changchien & Craven, 1977, 1978; Bogdanov et al., 1978; Mackie & Zimmermann, 1978; Allen & Wong, 1979b).

We have undertaken a systematic study of the molecular mechanism of ribosomal self-assembly in vitro. Our initial approach was to compare the conformations of the ribosomal proteins and RNA free in solution and those bound in the ribosomal particles under the conditions of reconstitution. It was shown that, in general, ribosomal proteins possess unique conformations free in solution which are susceptible to changes in environmental parameters and which are different from the

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[1] Abbreviations used: RI, reconstituted intermediate; RI*, heat-activated reconstituted intermediate; TP 30, total proteins extracted from the 30S subunit.

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conformations of proteins bound in the ribosome (Allen & Wong, 1978a). The hydrodynamic shape and conformation of the 16S RNA have been shown to be about the same free in solution and bound in the 30S subunit; only subtle conformational changes are observed upon binding of proteins to form the 30S subunit (Allen & Wong, 1978b). The next phase was to decipher the subtle conformational changes of the RNA and the complex conformational changes of the proteins during the successive steps of in vitro ribosome assembly. The 16S RNA was shown to undergo various subtle conformational changes during assembly by difference CD and difference UV absorption spectra (Dunn & Wong, 1979a). The conformational changes of the proteins during the assembly process was investigated by difference CD and chemical iodination (Dunn & Wong, 1979b). Complex changes occur in many proteins during various stages of in vitro assembly.

The present communication reports results of physical–chemical measurements which attempt to answer the following questions: (1) What are the hydrodynamic shape, conformation, and stability of the RI and RI*? (2) How do these intermediate particles compare with the 16S RNA on the one hand and the 30S subunit on the other? (3) What is the molecular nature of the heat activation of RI to RI* and its reversibility?

Materials and Methods

Buffers. The following buffers were used: TMK_{50}, 10 mM Tris-HCl, 20 mM $MgCl_2$, and 50 mM KCl, pH 7.6; TMK_{360}, 10 mM Tris-HCl, 20 mM $MgCl_2$, and 360 mM KCl, pH 7.6; TMK_{360} plus β-ME, TMK_{360} plus 6 mM β-mercaptoethanol; TM buffer, 30 mM Tricine and 20 mM magnesium acetate, pH 8.0; buffer III, 90 mM Tricine, 1.2 M KCl, and 60 mM magnesium acetate, pH 7.4; 30/50 buffer, 10 mM Tris-HCl, 1 mM $MgCl_2$, and 100 mM KCl, pH 7.4.

Preparation of Ribosome, Subunits, 16S RNA, and TP 30. E. coli MRE 600 cells were grown as described earlier (Wong et al., 1974). 70S ribosomes were prepared according to the procedure of Nomura and co-workers (Traub et al., 1971) with slight modifications (Wong et al., 1974). To obtain 30S subunits, the 70S ribosome sample was first dissociated into subunits by exhaustive dialysis against a 30/50 buffer overnight, and the subunits were separated in a Beckman Ti-15 zonal rotor by using a hyperbolic gradient extending from 7.4 to 38.0% sucrose according to the method of Eikenberry et al. (1970) with minor modifications (Allen & Wong, 1979a).

16S RNA was prepared by the acetic acid–urea extraction method (Hochkeppel et al., 1976). The detailed procedures used have been described earlier (Dunn & Wong, 1979a). TP 30 was extracted following essentially the acetic acid method of Hardy et al. (1969).

*Preparation of the Reconstituted 30S Subunit and the Reconstituted Intermediates, RI and RI**. Reconstitution of the 30S subunit and other RNA–protein complexes was carried out by using essentially the method of Traub & Nomura (1969) with the modification of Hochkeppel et al. (1976). The total 30S ribosomal protein sample was dialyzed against TMK_{360} and 6 mM β-mercaptoethanol just prior to reconstitution. The concentration of protein used was 475 μg/mL. The molar ratio of individual 30S proteins to 16S RNA was calculated on the basis of individual molecular weights and the assumption that 5 absorbance units at 230 nm correspond to 1 mg/mL protein (Hochkeppel et al., 1976). The concentration of 16S RNA was 560 μg/mL. The concentration of RNA was calculated by assuming that $\epsilon_{260nm}^{0.1\%} = 22.3$ (Stanley & Bock, 1965). The appropriate volumes of protein and RNA in TMK_{360} buffer were mixed to give a twofold molar excess of TP 30. The mixture was incubated for 45 min at 40 °C to form the reconstituted 30S subunit. After cooling the sample to 5 °C, the mixture was centrifuged for 15 min at 12000g to remove any precipitated material. The supernatant was then layered over 10 mL of TMK_{360} containing 12% sucrose and centrifuged for 15 h at 35 000 rpm in a Beckman Type 42.1 rotor (95000g). The 30S proteins which were bound to 16S RNA were then extracted and characterized by two-dimensional polyacrylamide gel electrophoresis as described (Dunn & Wong, 1979a). The reconstitution intermediate (RI) particles were formed by incubating 16S RNA and TP 30 for 30 min in TMK_{360}. These particles were purified by pelleting in 12% sucrose for 15 h at 35 000 rpm and analyzed as described above. The RI were then activated to RI* by incubation in TMK_{360} for 15 min at 37 °C.

Sedimentation Velocity. Sedimentation coefficients were determined by using a Beckman Model E analytical ultracentrifuge equipped with Schlieren optics. A 30-mm aluminum single-sector centerpiece was used with an An-E rotor. Concentrations for the RI and RI* particles ranged from 0.4 to 1 mg/mL, and measurements were obtained at 5 °C. The results for the RI particle were obtained during reconstitution whereas those for the RI* particle were obtained from heat activation of isolated RI. The observed sedimentation coefficients were corrected for the density and the viscosity of water at 20 °C by using the viscosity and density of the TMK_{360} buffer at 5 °C (1.499 cSt and 1.0194 g/mL, respectively). The partial specific volume for the RI and RI* particles was assumed to be 0.594 mL/g. The frictional coefficient ratio, f/f_{min}, was calculated from the sedimentation coefficient by using the equation

$$f/f_{min} = \frac{(4/3)^{1/3}}{6\eta(\pi N)^{2/3}} \frac{(1 - \bar{v}\rho)M_r^{2/3}}{\bar{v}^{1/3}s}$$

where M_r is the molecular weight, $\bar{v}$ is the partial specific volume, ρ and η are the density and viscosity of the solvent, respectively, and N and s are Avogadro's number and the sedimentation coefficient, respectively. The effective hydrodynamic radii, R_{sed}, for the RI and RI* particles were calculated by using the equation (Tanford, 1961)

$$R_{sed} = M_r(1 - \bar{v}\rho)/(6\pi\eta Ns)$$

where the symbols are the same as those defined above.

Melting Studies. Melting studies were carried out with the 16S RNA, RI particle, and 30S subunit using UV absorption and CD. UV hyperchromicity was recorded by using a Cary 118 CX double-beam spectrophotometer equipped with a thermally isolated compartment and temperature-regulated cell holders which were thermostated by a Lauda K2-R circulating water bath. CD studies were performed with a JASCO J-20 spectropolarimeter furnished with a jacketed cell holder made from an aluminum block with constant temperature water circulating from a Lauda K2-R water bath. Calibration curves were established for both instruments which corrected for the temperature difference between the solution in the cuvette and the circulating water bath. Silicone oil was layered on top of the samples to prevent evaporation during heating. All samples were dialyzed against TMK_{360} before measurements, and their initial absorbances at 260 nm were varied from 0.5 to 0.9 at 25 °C. Melting studies were carried out by raising the temperature in 5 °C increments. After reaching the desired temperature, each sample was allowed to equilibrate for 15 min before the absorbance or ellipticity was recorded. The UV hyperchromicity melting profiles were

Table I: Hydrodynamic Properties of the 16S RNA, RI, RI*, and 30S Subunit in TMK_{360}

	$s_{20,w}$[a] (S)	f/f_{min}[b]	R_{sed}[c] (A)	$\bar{\nu}$[d] (mL/g)
16S RNA	21.1 ($s^0_{20,w}$)[e]	2.16	113	0.577[f]
RI particle	29.4 ($s_{20,w}$)	1.55	104	0.597
RI* particle	26.5 ($s_{20,w}$)	1.96	115	0.597
30S subunit	32.6 ($s^0_{20,w}$)[e]	1.55	106	0.601[g]

[a] Sedimentation coefficient. [b] Frictional coefficient ratio. [c] Effective hydrodynamic radius. [d] Partial specific volume. [e] Allen & Wong (1978b). [f] Ortega & Hill (1973). [g] Hill et al. (1969).

expressed in terms of percent hyperchromicity at 260 nm according to the equation

$$\% \text{ hyperchromicity} = \frac{A_T - A_{25°C}}{A_{25°C}} \times 100$$

where A_T is the absorbance measured at the temperature in question and $A_{25°C}$ is the absorbance at 25 °C. The CD melting profiles were represented in terms of percent decrease in ellipticity at 265 nm.

Difference CD. Near- and far-UV CD studies were carried out to monitor the changes in conformation of the 16S RNA and the RI proteins during the RI → RI* transformation by heating the RI particle from 5 to 37 °C. In addition, the heating of the 16S RNA and the ribosomal proteins alone was studied. The reversibility of these reactions was also observed. Typically, a CD spectrum of the sample was obtained at 5 °C. The sample was then heated to 37 °C and incubated for 30 min, and another spectrum was recorded. To ascertain the reversible nature of the temperature effect, the sample was cooled to 5 °C and equilibrated for 30 min before recording another spectrum. Since at present there is no convenient way of accurately determining the concentration of the RI particle, the results are expressed in terms of relative ellipticity in units of degree centimeter^{-1} according to the equation: relative ellipticity = Θ_{obsd}/l, where Θ_{obsd} is the observed ellipticity in degrees and l is the pathlength in centimeters. The spectropolarimeter was purged with pure nitrogen gas during all measurements and routinely calibrated with *d*-10-camphorsulfonic acid (Cassim & Yang, 1969).

Miscellaneous. The concentrations of the 16S RNA and 30S subunit were determined by UV absorption at 260 nm using extinction coefficients of 22.3 (Stanley & Bock, 1965) and 14.8 (Hill et al., 1969), respectively. The concentration of the RI particle was estimated by determining the concentration of the 16S RNA and then assuming that there are 218 000 g of protein associated with each mole of 16S RNA. The concentration of the total 30S protein was ascertained by using an extinction coefficient of $A_{230nm}^{0.1\%} = 5$ (Hochkeppel et al., 1976).

Water was doubly distilled, deionized, and passed through a column of organic remover. All pH measurements were obtained at room temperature with a Model PHM64 Research pH meter equipped with a combined glass electrode (GK2302C). Reagent-grade chemicals were used throughout.

Results

Preliminary Hydrodynamic Studies of the RI and RI Particles.* The $s_{20,w}$ values for the RI and RI* particles have been obtained in the reconstitution buffer at 5 °C and are listed in Table I. They are intermediate between the values for the 16S RNA and the 30S subunit. The sedimentation velocity result for the RI particle was obtained in the presence of TP 30. Isolated RI particle purified by pelleting in 12% sucrose has a low solubility in TMK_{360}, thus preventing its measurement. Heating the solution increases the solubility but simultaneously converts RI particles to RI* particles.

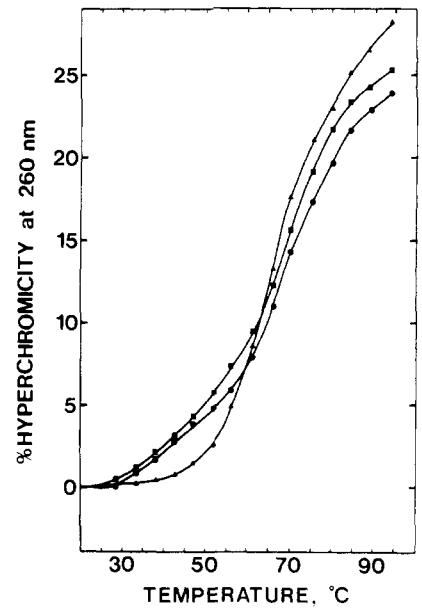

FIGURE 1: UV hyperchromicity melting profiles of 16S RNA (circles), the RI* particle (squares), and the 30S subunit (triangles). Conditions: TMK_{360} at pH 7.6. The percent hyperchromicity is determined at 260 nm.

The $s_{20,w}$ value obtained here for the RI particle is significantly higher than that reported by Traub & Nomura (1969). Their value of 21 S was obtained, however, by using sucrose density gradient centrifugation and assuming a value of 16 S for the marker ribosomal RNA. As shown in Table I, the $s^0_{20,w}$ for 16S RNA in the reconstitution buffer is 21.1 S (Allen & Wong, 1978b). Hill has independently obtained the same value under this condition (Hill, personal communication). The $s_{20,w}$ value for the RI* particle is less than that for the RI particle. This change in sedimentation coefficient indicates a tranformation to a more elongated shape for the RI* particle, which is also reflected by the frictional coefficient ratios and effective hydrodynamic radii shown in Table I. A similar observation was made by Held & Nomura (1973).

A problem remains with the hydrodynamic measurement. Although both the RI and RI* have been isolated, the two particles are likely to be heterogeneous in that not all particles contain a complete complement of all 15 proteins as shown by the two-dimensional gel electrophoresis. On the other hand, the measurement of RI in the presence of TP 30 appears to show rather homogeneous particles, probably due to the equilibrium with reversibly binding proteins. Thus, the comparison of hydrodynamic properties of the RI and RI* particles may not be completely valid but rather represents an approximation.

Thermal Stabilities of the 16S RNA, RI Particle, and 30S Subunit.* We have compared the thermal stability of the RI* particle to that of the 16S RNA and 30S subunit. Since the RI converts to RI* readily upon heating to 37 °C, no comparison between the two reconstitution intermediate particles is possible. The melting profiles were obtained by two different methods. UV hyperchromicity was used to monitor the extent and changes of base stacking/pairing, while CD reflects changes in asymmetric structural elements in the RNA upon melting.

The UV absorption melting profiles for the 16S RNA, the RI*, and the 30S subunit are shown in Figure 1. The melting curves were obtained under strictly controlled experimental

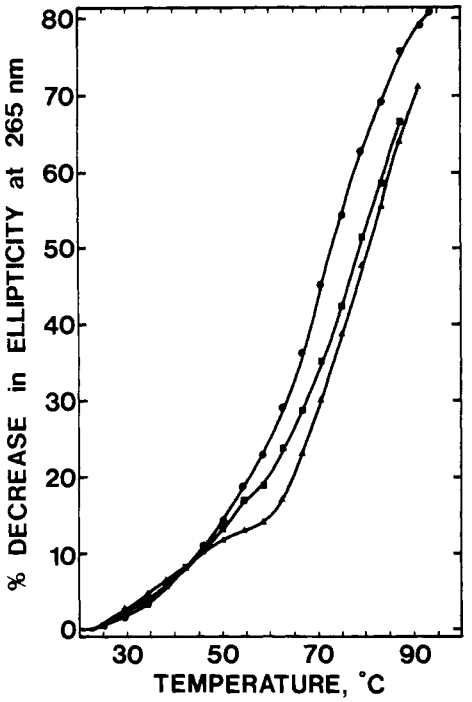

FIGURE 2: CD melting profiles of 16S RNA (circles), the RI* particle (squares), and the 30S subunit (triangles). Conditions: TMK_{360} at pH 7.6. The percent decrease in ellipticity is determined at 265 nm.

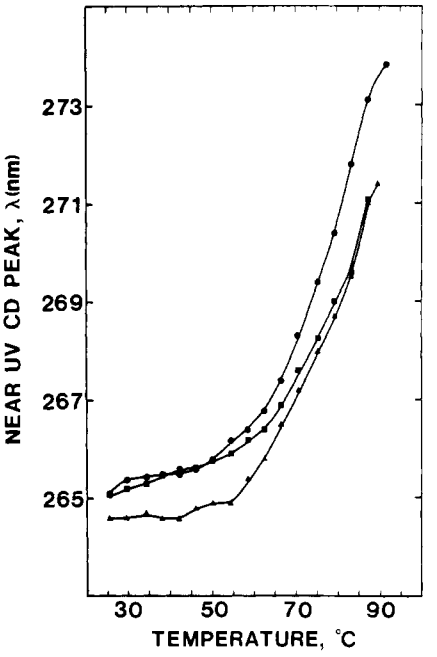

FIGURE 3: CD red-shift melting profiles of 16S RNA (circles), the RI* particle (squares), and the 30S subunit (triangles). Conditions: TMK_{360} at pH 7.6. The wavelength position of the near-UV peak is plotted.

conditions as described above; thus, relative differences among these three samples are genuine. The temperature range for these studies extends from 25 to 94 °C. However, as seen from Figure 1, none of these melting profiles clearly shows a plateau region at high temperature range. Thus, interpretation and conclusions on the amounts of base pairing/stacking in each of these states of the 16S RNA are not warranted. Despite this limitation, a number of significant observations can be made which are relevant to the stability of the 16S RNA at different states of assembly. A sigmoidal profile with gradual increase in absorbance at 260 nm was obtained for the free 16S RNA. A very similar profile was obtained for the RI*, but with the entire curve shifted systematically to lower temperatures (~2–3 °C lower). The melting profile for the 30S subunit differs drastically from those of the 16S RNA and the RI*. A large stabilization accompanying a sharp increase in cooperativity was observed on the lower portion of the profile below ~65 °C. Above 65 °C, a destabilization is observed compared to the RI*. At 94 °C, the percentages of hyperchromicity which represent the loss of relative amounts of base stacking/pairing upon heating are ~24, ~25, and ~28% for the 16S RNA, the RI*, and the 30S subunit, respectively.

The melting profiles for the 16S RNA, RI*, and 30S subunit have also been studied by the decrease in ellipticity of the near-UV CD and are shown in Figure 2. These curves reflect essentially the loss of asymmetric structural elements in the 16S RNA free in solution or in the RI* and 30S particles. Since the profiles do not show plateau areas at high temperature, and there is no evidence that a completely unwound RNA state is attainable, calculations and comparisons of the amounts of asymmetric conformation in the 16S RNA, RI*, and 30S subunit are again unwarranted. However, one can conclude that, on a relative scale, 16S RNA loses ~75% of its asymmetric structure at 87 °C, as compared with ~67 and ~63% for the RI* and 30S subunit, respectively, at the same temperature. Examination of the CD melting profiles reveals a gradual sigmoidal change through the entire temperature range studied (25 to ~90 °C). The binding of RI proteins to the 16S RNA to form the RI particle results in stabilization of the RNA conformation above 45 °C and renders a biphasic profile. Similar effects are observed for the 30S subunit with a more pronounced biphasic melting curve.

The difference in the magnitude of change observed by the hyperchromicity and the decrease in ellipticity methods used to monitor the melting process is quite striking. In the case of UV absorbance, the greatest percent hyperchromicity observed was ~28%, but, with CD, the largest change in ellipticity was ~76%.

In addition to monitoring the melting process by hyperchromicity and the decrease in ellipticity, the wavelength shift of the 265-nm CD peak was studied as a function of temperature and is shown in Figure 3. It has been suggested (Hashizume & Imahori, 1967) that a red shift of the near-UV CD peak of polyribonucleotides is indicative of base unpairing. As shown in Figure 3, both free 16S RNA and RI* have less base pairing than the 30S subunit below 65 °C. However, above ~65 °C the RI* appears to approach the melting curve of the 30S subunit. At ~90 °C, the RI* and 30S subunit are red-shifted ~2 nm less than the 16S RNA. This suggests that the bound proteins in the RI* and 30S subunit stabilize a significant amount of the base pairings as compared to the 16S RNA. This observation actually reinforces the conclusions from the hyperchromicity melting profiles at high temperature that the loss of RNA secondary structure is stabilized by the binding of proteins in the RI* and 30S subunit. The additional split proteins bound in the 30S subunits do not appear to stabilize further the base pairing of the 30S subunit when compared to the RI*.

Two additional features of the melting profiles monitored by a red shift of the 265-nm CD peak are observed. First, all these melting transitions occur at significantly higher temperatures than those monitored by hyperchromicity and the decrease in ellipticity. Second, the subtle discontinuity of the lower temperature portions of the melting profiles, especially the 30S profile, may represent distinct separable melting

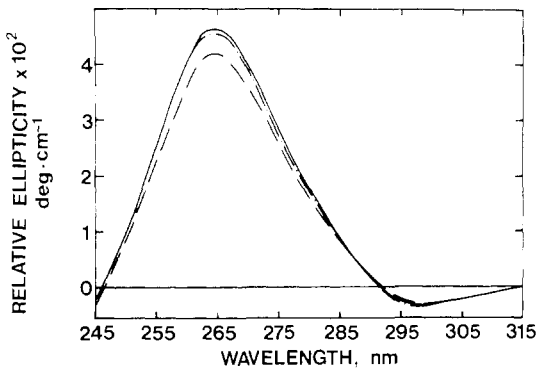

FIGURE 4: Near-UV CD spectra of the 16S RNA at 5 °C (solid curve), heated to 37 °C (dashed curve), and then cooled to 5 °C (broken curve). Conditions: TMK_{360} at pH 7.6.

transitions. Both observations have also been made by Phillips & Bobst (1972) on the melting of the R17 phage RNA.

Further Studies on Conformational Changes of Proteins and RNA during the Heat Activation of RI to RI and the Reverse Process.* It has been shown earlier that, during the assembly of the 30S subunit, the heat activation of the RI to RI* results in conformational changes of the 16S RNA and of the proteins. The former was shown to be mostly reversible and the latter was shown to be completely irreversible (Dunn & Wong, 1979a,b). We have further investigated these conformational changes by comparing the heating and cooling of RI and RI* particles and those of the RI and the reconstituted 30S subunit. Identical results were obtained. The far-UV CD difference spectra indicate that, upon heating to 37 °C, the ellipticity at 222 nm, which reflects conformational changes of proteins, decreases significantly and remains the same when the RI* is cooled back to 5 °C. On the other hand, the decrease in ellipticity at 265 nm in the difference CD spectra which originates from the RNA alone is mostly reversible (~75%) upon cooling.

To understand further the reversible nature of the temperature-dependent changes in the RNA and protein components during the RI → RI* transformations, similar heating and cooling experiments were done with the free 16S RNA and the free ribosomal proteins. Figure 4 shows the changes which occur in the 16S RNA as a result of heating to 37 °C. Upon cooling of the heat-activated RNA back to 5 °C, we found that the ellipticity at 265 nm is almost completely reversed.

The far-UV CD spectra in Figure 5 show the effect of heating the ribosomal proteins alone. In this case the decrease in ellipticity around 222 nm which accompanies an increase in temperature is completely reversible upon cooling when the proteins are not associated with the 16S RNA. This indicates that the irreversible change observed at 222 nm for the RI* must be dependent on the interaction of the ribosomal proteins with the 16S RNA.

Discussion

The preliminary hydrodynamic results suggest that as the free 16S RNA changes states by proceeding through the RI particle upon protein binding, then the RI* particle upon heat activation, and finally the 30S subunit, the shape-dependent hydrodynamic properties appear to become less elongated and/or more compact, then more asymmetric and/or less compact, and finally less asymmetric and/or more compact. The similarity of the RI particle and the 30S subunit is consistent with the electron microscopic results of Vasiliev et al. (1977), who have shown that protein-deficient derivatives

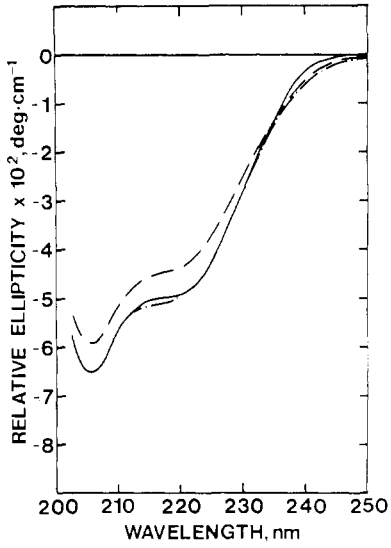

FIGURE 5: Far-UV CD spectra of the total 30S ribosomal protein at 5 °C (solid curve), heated to 37 °C (dashed curve), and then cooled to 5 °C (broken curve). Conditions: TMK_{360} at pH 7.6.

of 30S ribosomal subunits are "compact" and retain the main morphological features of the intact 30S subunit.

The exact interpretations of the melting studies are rather difficult at this time, because we still do not know the precise nature of the conformational changes monitored by each of the three parameters. However, some interesting observations can be made. The melting of the RI* is a complex process involving probably many conformational changes throughout the entire temperature range studied. The transition is broad and not highly cooperative. Similar observations are made for the 16S RNA and the 30S subunit except for the melting of the latter analyzed by hyperchromicity. This indirectly suggests the complexity of the 16S RNA conformation at its various assembly steps. The multistaged nature of the melting process is reinforced by the multiple parameter test. The three parameters used to follow the melting originate from different conformational aspects of the RNA and the fact that the three corresponding melting profiles of RI* are nonidentical suggest that the melting process of RI* is a complex process involving conformational intermediates. Similar conclusions can also be made for the 16S RNA and 30S subunit.

The melting behavior of the RI*, in general, differs significantly from the 16S RNA and the 30S subunit. It is very similar to 16S RNA at the low temperature range below ~50 °C but closer to the 30S subunit at high temperature ranges above 80 °C. In the middle of the transition, between approximately 50 and 80 °C, the RI* behavior is intermediate between that of the 16S RNA and that of the 30S subunit.

One interesting aspect of the CD melting results is that both RI* and the 30S subunit appear to be stabilized by the bound proteins and are more resistant to conformational disruption by high temperature.

Another interesting observation of the CD melting transition profiles is the biphasic nature of the curves for the RI* particle and the 30S subunit which is induced by the binding of proteins. This phenomenon suggests the existence of two distinct thermal-stability domains in the RI* particle and the 30S subunit as a result of the binding of proteins. In the case of the 30S subunit, as compared to the RI* particle, it is observed that the complete complement of ribosomal proteins causes the biphasic nature of the transition to appear even more dramatic. The existence of two stability domains has also been shown by the results on the denaturation of the 30S subunit

by ethylene glycol (Wong et al., 1976; Fox et al., 1978).

The conformational changes of the 16S RNA and the RI proteins during the heat activation of RI to RI* appear to originate from the RNA and the proteins alone, since similar conformational changes are observed for the free RNA and the free proteins in solution. However, in the RI* the complete irreversibility of the protein conformational change and the small irreversibility of the RNA conformation upon cooling can be attributed to the protein–RNA interactions in the RI* particles, since the respective conformational changes for the proteins and the 16S RNA alone are completely or almost completely reversible.

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