ON THE EQUILIBRIUM OF THE ASSOCIATION–DISSOCIATION REACTION OF RIBOSOMAL SUBPARTICLES AND ON THE EXISTANCE OF THE SO-CALLED '60 S INTERMEDIATE' ('SWOLLEN 70 S') DURING CENTRIFUGATION OF THE EQUILIBRIUM MIXTURE

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1. Introduction

A sedimentation analysis of NH_4Cl-washed 70 S ribosomes or 50 S–30 S couples obtained by re-association of isolated ribosomal subparticles (i.e., non-translating uncharged ribosomes) often reveals a component sedimenting not at 70 S, but essentially slower. Thus, a 60 S component instead of the 70 S was observed during zonal centrifugation of uncharged 50 S–30 S couples in a sucrose gradient [1, 2]. In other cases a 60 S component was observed simultaneously with the 70 S during sucrose gradient centrifugation as an intermediate stage in the dissociation of the 70 S ribosomes into the 50 S and 30 S subparticles [3, 4]. Recently a 60 S component was reported during sucrose gradient centrifugation of 50 S–30 S couples precharged with poly U and deacylated tRNA (in the ionic conditions used, the 50 S–30 S couples without such a precharging were evidently so unstable that during the zonal centrifugation they were not observed) [5]. Reports also appeared on a 50 S component during centrifugation of the 50 S–30 S couples in conditions when they are on the verge of dissociation [6, 7]. Most authors unreservedly come to the conclusion that a special conformationally changed 70 S ('loosened' or 'swollen 70 S', '60 S intermediate particle' etc.) has been revealed [3–7]. Only in two cases has a reservation been made that the apparent 60 S component may represent not real particles, but may be the result of a reversible dissociation and re-association of 70 S ribosomes in the course of sedimentation [1, 2].

In the present communication the sedimentation behaviour of uncharged 50 S–30 S couples in the analytical ultracentrifuge at 10 mM Mg^{2+} and 160 mM NH_4^+ is reported. It has been observed that with a decrease of the ribosomal concentration the 50 S–30 S couples exhibit a progressing tendency to dissociate into separate 50 S and 30 S subparticles, according to the analysis at relatively high sedimentation speeds. At relatively low sedimentation speeds the same ribosomal suspensions displayed a single component with a sedimentation coefficient from 65 S to 52 S depending on the ribosome concentration. A conclusion is made on the existence of a dynamic equilibrium

$$50\text{ S}–30\text{ S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} 50\text{ S} + 30\text{ S}.$$

2. Materials and methods

Preparations of both NH_4Cl-washed 70 S ribosomes and 50 S–30 S couples formed by associating washed isolated 50 S and 30 S subparticles were used in the experiments. The preparations were obtained from *Escherichia coli*, strain MRE-600.

NH_4Cl-washed ribosomes were prepared by 4-fold recentrifugation of the ribosomes from 1 M NH_4Cl with 10 mM $MgCl_2$ [8]. Prior to the experiment, the ribosomes were dialyzed against a buffer containing 10 mM $MgCl_2$, 160 mM NH_4Cl and 10 mM tris-HCl, pH 7.2, and the suspension left overnight in the cold.

The 50 S and 30 S ribosomal subparticles were prepared as described previously [9]. The 50 S and 30 S subparticle suspensions were dialyzed separately against a buffer containing 10 mM $MgCl_2$, 160 mM NH_4Cl and 10 mM tris-HCl, pH 7.2, then equimolar amounts of 50 S and 30 S subparticles were mixed and the mixture left overnight, in the cold, for association.

The different dilutions of ribosomes were prepared from the same initial mixture. The concentration of ribosomes was measured with the SF-4 spectrophotometer (USSR), taking 1 optical unit at 260 nm as corresponding to 66 μg of ribosomes. Suspensions with ribosomal concentrations of 4 mg per ml, 1.3 mg per ml, 0.4 mg per ml, 0.1 mg per ml, and 0.04 mg per ml were utilized for analysis in the ultracentrifuge. Sedimentation analysis was done on the Spinco model E (Beckman) ultracentrifuge equipped with schlieren and ultra-violet absorption optics, using 3 mm, 12 mm or 30 mm analytical cells depending on the concentration of ribosomes. The rotor speeds were 42,040 rpm (high speed) and 12,590 rpm or 13,410 rpm (low speed) at 20°.

3. Results

3.1. *Does an observable dynamic equilibrium between 50 S–30 S couples and free subparticles exist?*

Mixing of uncharged 50 S and 30 S subparticles under appropriate ionic conditions (at a high enough Mg^{2+} concentration) results in the formation of 50 50 S–30 S couples considered as uncharged ribosomes. Ribosomes washed with 1 M NH_4Cl are also usually regarded as uncharged 70 S ribosomes. The presence of such 50 S–30 S couples as a dominating component at 10 mM Mg^{2+}–160 mM NH_4^+ may be seen in fig. 1A.

The first question which arises is the following: is there an observable dynamic equilibrium between couples and subparticles, i.e., 50 S–30 S $\rightleftharpoons$ 50 S + 30 S, in such a suspension of uncharged ribosomes? This question can be answered by examining the ratio between the components at different concentrations of ribosomes in the suspension: in the case of an existing equilibrium dilution of the suspension (at constant ionic conditions) must decrease k_2 without changing k_1, i.e. shift the equilibrium towards dissociation; thus, dilution must increase the proportion of 50 S and 30 S subparticles. Table 1 represents data illustrating the decrease of the fraction of 50 S–30 S couples (65 S–68 S component) upon dilution of the suspension from 4 mg per ml to 0.04 mg per ml, according to analysis of the suspensions in the ultracentrifuge at 42,040 rpm. Fig. 1 shows sedimentation patterns of the same sample of the re-associated ribosomes at 0.4 mg per ml, 0.1 mg per ml and 0.04 mg per ml (A, B and C, respectively). The decrease of the proportion of the couples upon the dilution is seen. From the concentration dependence of proportion between couples and subparticles the conclusion can be drawn on the existence of a noticeable dynamic equilibrium

$$50\text{ S}–30\text{ S} \rightleftharpoons 50\text{ S} + 30\text{ S}.$$

3.2. *Can the equilibrium ribosomal mixture be observed as a single component?*

If a dynamic equilibrium exists then the analysis of such a suspension in the ultracentrifuge may give a sedimentation picture, whose interpretation is not simple [10]. First of all, in the case of the rates of forward and reverse reactions large enough in comparison with sedimentation velocities the components of the equilibrium mixture will not be separated in the ultracentrifuge but will sediment as a single component with an intermediate sedimentation coefficient.

In the above experiments, at 42,040 rpm, a separation of boundaries of the 50 S–30 S couples and the 50 S and 30 S subparticles can be seen (fig. 1, A–C). This means that the transport (sedimentation) velocity in the ultracentrifuge (about 0.05 cm/min for the 50 S–30 S couple at 42,040 rpm) is large enough in comparison with the rates of the forward and reverse reaction.

Using the same ribosomal suspensions repeated experiments were made where the transport velocity was decreased approximately ten-fold by slowing the rotor rotation speed to 12,590 rpm or 13,410 rpm. It is seen that in this case the same ribosomal suspensions displayed a single component with an intermediate sedimentation coefficient value (fig. 1, A′–C′). In the case of the suspension with a 0.1 mg per ml ribosomal concentration the sedimentation coefficient ($s_{20,w}$) of the component was found to be about 60 S (fig. 1, B′).

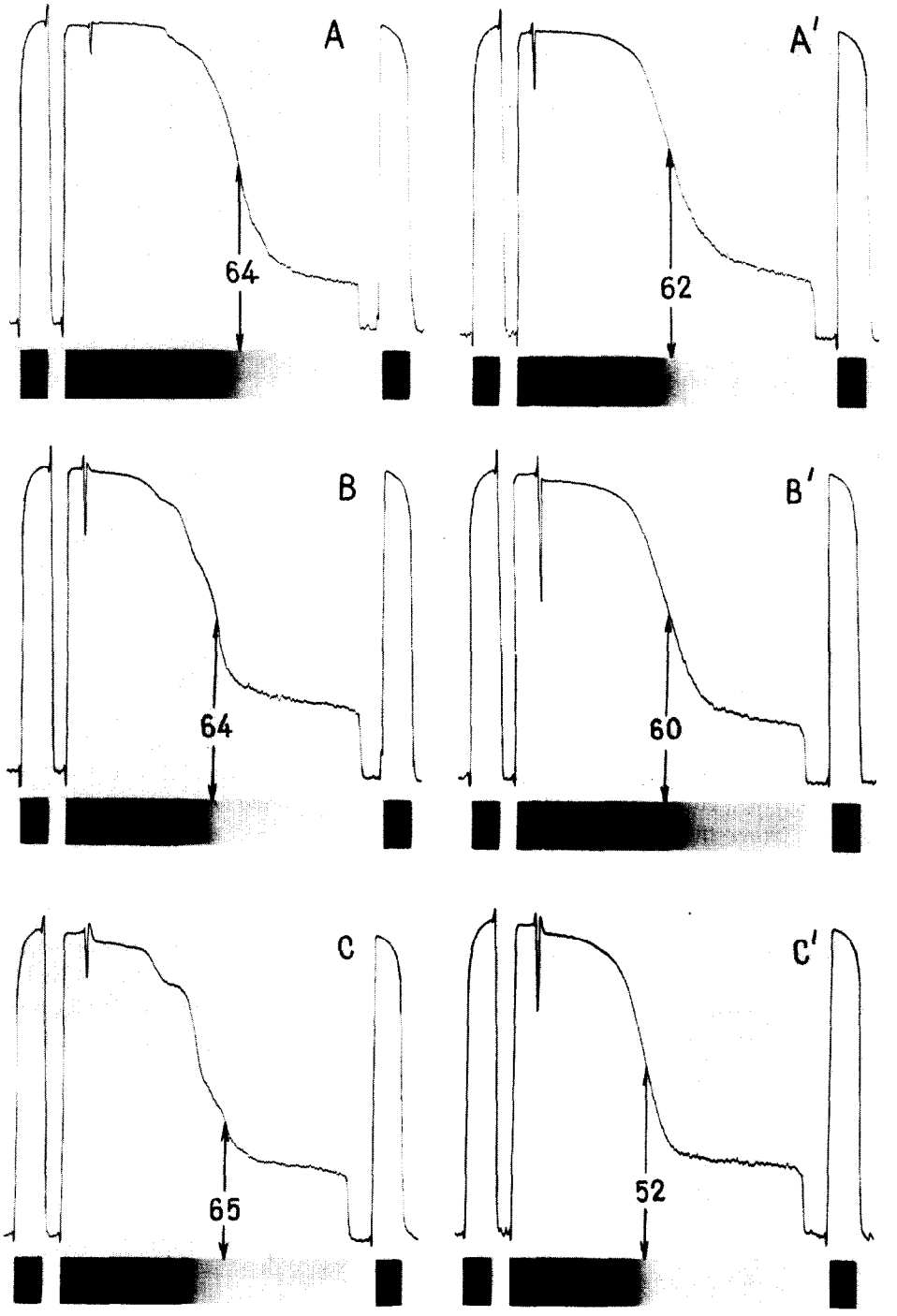

Fig. 1. Sedimentation patterns and their densitograms (in terms of light transmittance versus distance) of ribosomes obtained by re-association of 50 S and 30 S subparticles in 10 mM Mg^{2+}–160 mM NH_4^+. A, B and C: rotor speed 42,040 rpm; photographs taken approximately 8 min after attaining the speed. A′, B′ and C′: rotor speed 12,590 rpm; photographs taken approximately 150 min after attaining the speed. A and A′: 0.4 mg of ribosomes per ml (3 mm cell). B and B′: 0.1 mg of ribosomes per ml (12 mm cell). C and C′: 0.04 mg of ribosomes per ml (30 mm cell).

Table 1
Relative content of 50 S–30 S couples at different concentrations of ribosomal particles according to analytical ultracentrifugation data at 42,040 rpm (10 mM Mg^{2+}–160 mM NH_4^+).

Concentration of ribosomes (mg per ml)	NH_4Cl-washed ribosomes		Re-associated ribosomes	
	Sedimentation coefficient of couple boundary $s_{20,w}$	Fraction of couples (% %)	Sedimentation coefficient of couple boundary $s_{20,w}$	Fraction of couples (% %)
4	–	–	63.8	75 ± 4
1.3	65.7	66 ± 2	64.5	74 ± 4
0.4	67.4	63 ± 2	63.8	66 ± 3
0.1	68.1	52 ± 3	64.3	51 ± 4
0.04	67.6	42 ± 3	64.6	32 ± 5

The accuracy of $s_{20,w}$ measurements is within limits of ±1%.

Thus, at not very high transport (sedimentation) velocities in the ultracentrifuge the multicomponent equilibrium mixture 50 S–30 S $\rightleftharpoons$ 50 S + 30 S may be revealed as a single component.

3.3. *Does the apparent single component indeed not represent real 'intermediate' 60 S particles?*

During centrifugation of systems of reversibly interacting (rapidly associating–dissociating) particles, the shift of the equilibrium towards dissociation by dilution must lead to a decrease of the sedimentation coefficient of an apparent component [10]. In other words, if our apparent 60 S component is an unseparatable equilibrium mixture, then the sedimentation coefficient is the weight-average value of the sedimentation coefficients of couples and subparticles in this mixture; from this it follows that with dilution the sedimentation coefficient must decrease, while during the transition to more concentrated suspensions it must approach the sedimentation coefficient value of pure couples.

Table 2
Sedimentation coefficients of the apparent component at different concentrations of ribosomal particles according to analytical ultracentrifugation data at 12,590–13,410 rpm (10 mM Mg^{2+}–160 mM NH_4^+).

Concentration of ribosomes (mg per ml)	NH_4Cl-washed ribosomes	Re-associated ribosomes
	$s_{20,w}$	$s_{20,w}$
4	64.4	64.1
1.3	64.6	64.5
0.4	64.4	61.9
0.1	59.4	60.0
0.04	56.8	52.4

The accuracy of $s_{20,w}$ measurements is within limits of ±1.5%.

Analysis in the ultracentrifuge at low speeds (fig. 1, A′–C′ and table 2) shows that the apparent single component can have a sedimentation coefficient from 52 S to 65 S depending on the ribosomal concentration. Thus, dilution decreases the sedimentation coefficient of the '60 S component', while an increase in the concentration leads to an increase in its sedimentation coefficient, confirming with that expected for the equilibrium mixture 50 S–30 S $\rightleftharpoons$ 50 S + 30 S and not with that for real 60 S particles.

4. Discussion and conclusions

(1) At definite ranges of ionic conditions (for example at 10 mM Mg^{2+}–160 mM NH_4^+) a reversible dynamic equilibrium is observed between uncharged ribosomal 50 S and 30 S subparticles and their couples:

$$50\text{ S}–30\text{ S} \rightleftharpoons 50\text{ S} + 30\text{ S}.$$

As a result of this, the ratio between subparticles and couples (the couple fraction) in the given conditions is not constant and must strongly depend on the total concentration of ribosomal particles in the suspension; a dilution of the suspension must shift the equilibrium towards dissociation.

The rates of forward and reverse reactions of the observed dynamic equilibrium seem to be relatively not very rapid, in any case they are comparable with particle sedimentation velocities within the range

between 12,000 *g* (13,000 rpm) and 120,000 *g* (42,000 rpm).

(2) As the reaction rates in the described equilibrium are not high, sedimentation analysis of the equilibrium mixture *at high speeds of centrifugation* can mainly succeed in separating the components of the mixture (30 S, 50 S and 50 S–30 S couples). However, since during centrifugation some dissociation of 50 S–30 S couples does take place, then this must lead to: (a) a spreading of the moving boundary of couples towards a more slowly sedimenting material (formation of a 'tail'), and (b) a greater or lesser underestimation of the couple fraction observable by sedimentation analysis, in comparison with their real proportion in the equilibrium mixture. This must be true both for moving boundary sedimentation, and to an even greater extent, for zonal sedimentation in the sucrose gradient (up to the full dissociation of couples in the process of centrifugation).

Thus, the quantitative evaluation of the proportions of the components (fraction of couples) from the sedimentation pattern may be far from reflecting the real proportions of the components in the equilibrium mixture.

(3) Centrifugation of the equilibrium mixture *at relatively low sedimentation rates* may reveal a single component with a sedimentation coefficient intermediate between those of the couple and its subparticles (for example, 60 S). This apparent component is not a real class of particles, but reflects the sedimentation of an unseparatable equilibrium mixture (the couples dissociating and re-associating in the course of sedimentation).

In connection with these data it is necessary to be very cautious in interpreting any sedimentation pictures where components with intermediate (from 50 S to 70 S) sedimentation coefficients are revealed; in any case the observation of the 60 S zone or boundary in the ultracentrifuge does not warrant unreversed conclusions on the existance of 'intermediate particles', 'loosened' or 'swollen' 70 S, 'conformationally changed 70 S' etc., made by some authors [3–7].

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