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Apparent Changes in Ribosome Conformation during Protein Synthesis. Centrifugation at High Speed Distorts Initiation, Pretranslocation, and Posttranslocation Complexes to a Different Extent†

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ABSTRACT: We prepared three intermediates in protein synthesis: *initiation complex* (f2 bacteriophage RNA·ribosome complex with formylmethionyl-tRNA$_f^{Met}$ (fMet-tRNA) in the P site). Part of the initiation complex was converted into a *pretranslocation complex* (f2 bacteriophage RNA·ribosome complex with fMet-Ala-tRNA in the A site and discharged tRNA in the P site). Part of the pretranslocation complex was converted into a *posttranslocation complex* (f2 bacteriophage RNA·ribosome complex with fMet-Ala-tRNA in the P site, having the A site vacant). We found that the pretranslocation complex sediments faster in a sucrose gradient centrifuged at high speed (230,000*g*, hydrostatic pressure 1200 atm) than either the posttranslocation complex or the initiation complex. The sedimentation patterns (including the per cent recovery in the peak fractions) of the pretranslocation and posttranslocation complexes centrifuged at high speed did not seem to be altered when the amount of the ribosome complexes in the gradients were decreased ninefold. In view of this it is unlikely that the difference in sedimentation velocities between the two complexes during high-speed centrifugation is due to a difference between them in reversible dissociation. In a gradient centrifuged at low speed (51,500*g*, hydrostatic pressure 300 atm) the pretranslocation and the posttranslocation complexes sedimented together. A mixture of pre- and posttranslocation complexes was first partially resolved by centrifugation through a sucrose gradient at high speed, then the fractions of the gradient containing either or both of the complexes were mixed and concentrated, and finally, the resulting solution was applied to a sucrose gradient which was centrifuged at low speed. In these conditions the two complexes appeared to sediment with an equal velocity. These and other data indicate that centrifugation at high speed does not result in an irreversible change in the sedimentation characteristics of the complexes, *i.e.*, probably does not cause an irreversible loss of components from them. All these findings may be explained by the following assumptions: the large hydrostatic pressure prevailing during centrifugation at high speed distorts the conformation of the two complexes to a different extent, and this in turn accounts for the difference between the sedimentation velocities of the two complexes. Consequently the difference in sedimentation velocities between the pre- and the posttranslocation complexes manifested at high-speed centrifugation is no proof for a difference in conformation between the complexes under physiological conditions.

Recently we developed an assay for measuring ribosome movement along the mRNA[1] during protein synthesis (Gupta *et al.*, 1971; see also Thach and Thach, 1971). These served to establish that (1) one step of the ribosome is as expected three nucleotides long, and (2) ribosome movement is triggered by the same factor (S_2) and GTP which triggers translocation of peptidyl-tRNA from site A to site P on the ribosome. The study required the preparation of three intermediates

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[1] Abbreviations used are: Init, initiation complex; Pre, pretranslocation complex; Post, posttranslocation complex; rcf, relative centrifugal force; GMPPCP, 5′-guanylylmethylenediphosphonate.

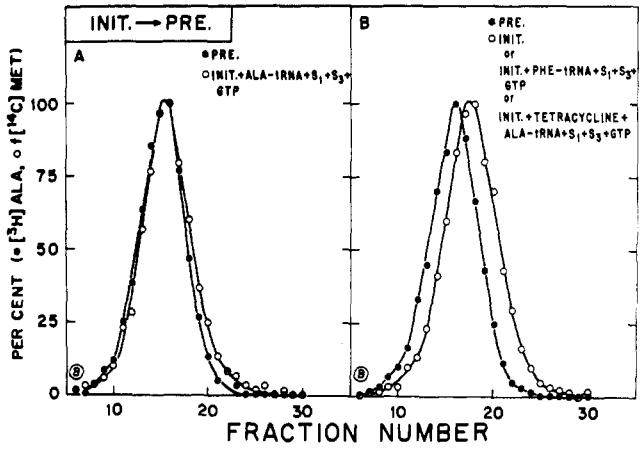

FIGURE 1: Difference between the sedimentation velocities of Init and Pre centrifuged at high speed. The conversion of the slower sedimenting form to the faster sedimenting form requires the same treatment as the conversion of Init to Pre. (A) f[^{14}C]Met-labeled Init (in 0.15 ml) was converted to Pre by incubation with 0.1 ml of Ala-tRNA·S_3·GTP complex solution (○, Init Ala-tRNA + S_1 + S_3 + GTP). The reaction mixture was mixed with 0.25 ml of "marker Pre" (●, Pre) at 0° prior to application to a linear sucrose gradient. Marker Pre contained [^{3}H]Ala and had been purified by gel filtration to separate S_2 and GTP from the ribosome complex. (B) The conditions were similar to those in A except that the Init was either (i) tested as such (○, Init) or (ii) was first supplemented with a Phe-tRNA·S_3·GTP complex solution in place of the Ala-tRNA·S_3·GTP complex solution (Init + Phe-tRNA + S_1 + S_3 + GTP) or (iii) tetracycline (final concentration 0.1 mM) was added to the Init before adding the Ala-tRNA·S_3·GTP complex solution (Init + tetracycline + Ala-tRNA + S_1 + S_3 + GTP). 0.2 ml of each of the samples was mixed with 0.25 ml of marker Pre (●, Pre) prior to application to a linear sucrose gradient. Each sucrose gradient was centrifuged at 40,000 rpm. The patterns of sedimentation of i, ii, and iii were indistinguishable.

in protein synthesis. Init[1] was obtained by binding a single ribosome to the coat protein initiation site of f2 bacteriophage RNA in the presence of GTP, initiation factors and the chain initiator fMet-tRNA$_f^{Met}$. Pre was prepared by reacting Init with the appropriate factors (S_1 and S_3), GTP and Ala-tRNA (alanine is the second amino acid of the coat proteins). In this reaction fMet-Ala-tRNA is formed. Post was obtained by reacting Pre with the appropriate factor (S_2) and GTP. This results in the release of discharged tRNA from the ribosome, the shift of fMet-Ala-tRNA from the A site to the P site, and the movement of the ribosome along the mRNA. As part of the above study, each complex was sedimented through a sucrose gradient centrifuged at high speed (180,000*g*). It was noted that the Pre sedimented faster than either the Init or the Post. Similar differences in sedimentation velocities among Pre, Post, and Init containing a synthetic mRNA (poly(U)) were reported by Schreier and Noll (1971) and Chuang and Simpson (1971). These differences were attributed to differences in conformation between the various ribosome complexes.

Recent publications reveal the need for caution in interpreting sedimentation patterns in which ribosomes with sedimentation coefficients between 70 and 50 S are found. According to Spirin (1971) such patterns may not necessarily reflect a real class of ribosomal particles with an unusual conformation but may result from the incomplete separation of an equilibrium mixture of ribosomes and subunits, dissociating and reassociating during centrifugation. Furthermore, Infante and his associates (Infante and Graves, 1971; Infante and Krauss, 1971; Infante and Baierlein, 1971) established that large hydrostatic pressure prevailing during high-speed centrifugation induces dissociation of free ribosomes.

The aim of our study was threefold: (1) to correlate the requirements for the conversion of the slow-sedimenting Init to a faster sedimenting complex with those needed for its conversion to the Pre; (2) to correlate the requirements for the conversion of the faster sedimenting Pre to a slower sedimenting complex with those needed for its conversion to the Post; (3) to study the causes of the difference in sedimentation velocities between the complexes.

Some of the results obtained were presented at the XI Latin American Symposium on Protein Synthesis and Nucleic Acids in La Plata in Nov 1971.

Experimental Section

Published procedures (Gupta *et al.*, 1971) were used in obtaining fMet-tRNA$_f^{Met}$ unlabeled or labeled with [^{14}C]-Met, Ala-tRNA labeled with [^{3}H]Ala or [^{14}C]Ala, ribosomes from *E. coli* and peptide chain elongation factors from *Bacillus stearothermophilus* (S_1 purified 50-fold, S_2 75-fold, and S_3 70-fold, a homogeneous protein). These factors are analogous to factors from *E. coli* and *Pseudomonas fluorescens*. S_1 corresponds to T_s and TI_s, S_2 to G and TII, and S_3 to T_u and TI_u (Lucas-Lenard and Lipmann, 1966; Ravel *et al.*, 1970).

Preparation of Init. One milliliter of the reaction mixture contained: 50 mM Tris-HCl (pH 7.4), 50 mM NH_4Cl, 6 mM 2-mercaptoethanol, 5 mM Mg^{2+}, 0.4 mM GTP, 5×10^{-4} mM fMet-tRNA$_f^{Met}$ unlabeled or labeled with [^{14}C]Met (265 Ci/mole) or [^{35}S]Met (1373 Ci/mole, this was used only in experiments on the effect of ribosome concentration on the sedimentation velocity), 30 A_{260} units of ribosomes, and 20 A_{260} units of f2 RNA. After incubation at 37° for 15 min the reaction mixture was cooled to 0°.

Preparation of AA-tRNA·S_3·GTP Complex. One milliliter of the reaction mixture contained: 50 mM Tris-HCl (pH 7.4), 50 mM NH_4Cl, 10 mM Mg^{2+}, 6 mM 2-mercaptoethanol, 0.05 mM GTP, 40 μg of S_1, 40 μg of S_3, and either 6 A_{260} units of Ala-tRNA carrying 54 pmoles of Ala residues/A_{260} unit labeled with either [^{3}H]Ala (2700 or 52,000 Ci per mole, this was used only in experiments on the effect of ribosome concentration on the sedimentation velocity), or [^{14}C]Ala (136 Ci/mole) or 18 A_{260} units of Phe-tRNA carrying 21 pmoles of unlabeled Phe residues/A_{260} unit.

Preparation of Pre. One volume of Init was mixed with 0.5 volume of AA-tRNA·S_3·GTP complex and incubated at 0° for 40 min.

Preparation of Post. Pre (0.25 ml; purified by gel filtration if so indicated) was supplemented with 0.2 mM GTP and 2.5 μg of S_2 and incubated at 37° for 5 min.

Purification of Pre and Post Preparations by Gel Filtration. Sephadex G-200 columns (volume 12.5 ml) were equilibrated with buffer A (50 mM Tris-HCl (pH 7.4), 50 mM NH_4Cl, 7 mM Mg^{2-}, and 6 mM 2-mercaptoethanol). Up to 1 ml of a Pre or Post preparation was applied to the column and eluted with buffer A at 0°. The fractions of the void volume containing the complex (as detected by measuring radioactivity or absorbancy) were pooled and used. It will be indicated if a complex preparation was purified by gel filtration.

Analysis of Complexes by Sedimentation through Sucrose Gradients. LINEAR GRADIENTS (Figures 1 and 2). Five to twenty per cent (w/v) linear sucrose gradients (volume 12.5 ml) were prepared in buffer A. Up to 1.2 ml of sample was applied to each. The gradients were centrifuged in the SB283

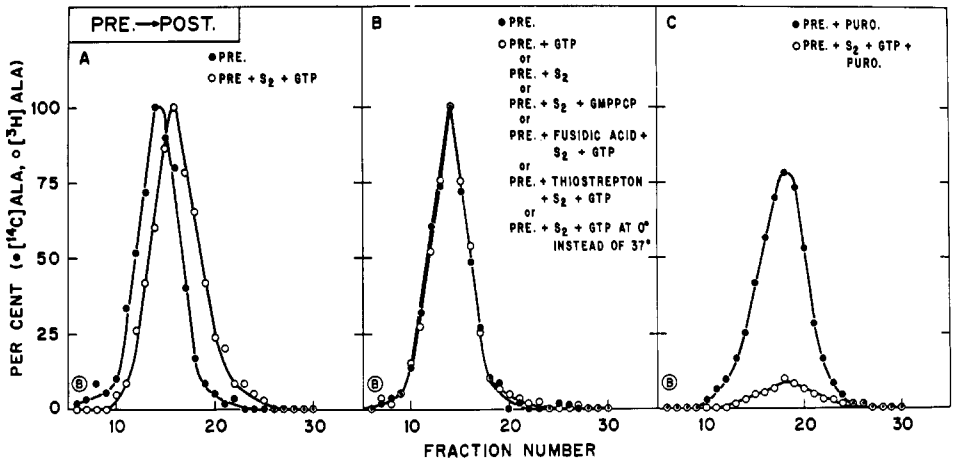

FIGURE 2: Difference between the sedimentation velocities of Pre and Post centrifuged at high speed. The conversion of the faster sedimenting form to the slower sedimenting form requires the same treatment as the conversion of Pre to Post. Marker Pre contained [^{14}C]Ala and had been purified by gel filtration. (A) Post was prepared by reacting 0.25 ml of Pre (labeled with [^{3}H]Ala) which had been purified by gel filtration, with GTP and S_2, at 37° (○, Pre + S_2 + GTP). The Post was mixed with 0.25 ml of marker Pre (●, Pre) prior to applying the reaction mixture to a sucrose gradient. (B) The samples (○, Pre + GTP) and (Pre + S_2) were prepared as in A except for the omission of S_2 from the first and GTP from the second. In the sample (Pre + S_2 + GMPPCP), 2 mM GMPPCP was substituted for GTP in the reaction mixture containing Pre + S_2. In the sample (Pre + fusidic acid + S_2 + GTP), 10^{-3} M fusidic acid was present in the reaction mixtures in which the Init was converted to Pre and in which the Pre was treated with S_2 and GTP. In the sample (Pre + thiostrepton + S_2 + GTP), 10^{-5} M thiostrepton was substituted for the fusidic acid. In the sample (Pre + S_2 + GTP at 0° instead of 37°) the Pre was incubated with S_2 and GTP at 0°. Each of the samples was mixed with an equal volume of marker Pre prior to application to a linear sucrose gradient. The patterns of sedimentation of the samples excepting marker Pre were indistinguishable. (C) The samples were prepared as in part A except that before centrifugation through a linear sucrose gradient the reaction mixture was made 2 mM in puromycin and incubated at 0° for 10 min. Each sucrose gradient was centrifuged at 40,000 rpm.

rotor of the IEC B60 centrifuge at 40,000 rpm, average rcf 180,000*g* at 2° for 3.6 hr.

ISOKINETIC GRADIENTS (Noll, 1969) (Figures 3 and 4). The mixing chamber used in preparing the gradients contained 12.8 ml of 5% (w/v) sucrose solution in buffer A. The reservoir contained 12.5 ml of 30.8% (w/v) sucrose solution in buffer A. Sedimentation was performed in the same rotor as above either at *high speed* (40,000 rpm, average rcf 180,000*g*, rcf between the positions of the peak fractions of the Pre and Post 230,000*g*, hydrostatic pressure at the same position

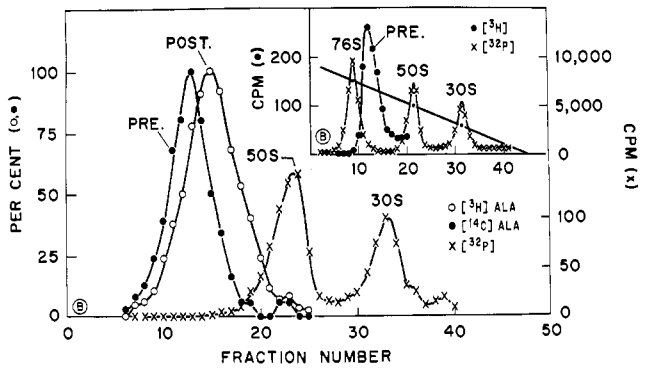

FIGURE 3: Determination of the difference between the sedimentation velocities of Pre and Post centrifuged at high speed. [^{3}H]Ala-labeled Post (0.3 ml) (see Figure 2) was mixed with 0.25 ml of [^{14}C]Ala-labeled Pre and 5 μl each of ^{32}P-labeled 30S and 50S ribosomal subunits. (These served as sedimentation velocity markers in the gradients.) The reaction mixture was analyzed by centrifugation at high speed through the isokinetic sucrose gradient. The insert shows an experiment in which 0.25 ml of [^{3}H]Ala-labeled Pre, which had been purified by gel filtration, was mixed with ^{32}P-labeled bacteriophage f2 (sedimentation coefficient 76 S) and ^{32}P-labeled 30S and 50S ribosomal subunits and centrifuged at high speed through the isokinetic sucrose gradient. The fractions were counted without filtration. The distances between the abscissa and the points of the intersect of the descending straight line with the imaginary lines parallel with the ordinate at the middle of the 76S, 50S, and 30S peaks are related to one another as 76 to 50 to 30. This verifies that the gradient was isokinetic, *i.e.*, the distance of sedimentation of each component was proportional to its S value.

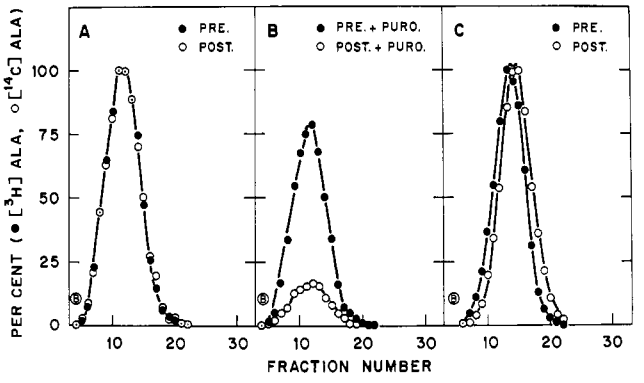

FIGURE 4: The difference between the sedimentation velocities of Pre and Post is manifested at high-speed but not at low-speed centrifugation. [^{3}H]Ala-labeled Pre was prepared by reacting Init with Ala-tRNA·S_3·GTP complex in the presence of 10^{-5} M thiostrepton. (The antibiotic was added to block translocation. It was established in separate experiments that (i) the sedimentation patterns of Pre and Post are not affected by the presence of thiostrepton, and (ii) Pre can be formed in the presence of thiostrepton, though at a slow rate.) Pre and [^{14}C]Ala-labeled Post were purified by gel filtration. A mixture of equal volumes of the two complexes (total volume 1 ml) was divided into aliquots i and ii. Aliquot i was *centrifuged at low speed* (20,000 rpm) *for 16.8 hr* through the isokinetic sucrose gradient. One half of each fraction of the gradient was counted (A). The second half was supplemented with 2 mM puromycin, incubated at 0° for 10 min to release the fMet-Ala residue from the Post), and counted (B). Aliquot ii *was stored* at 2° (*i.e.*, the temperature of the gradient during centrifugation) for *12.6 hr and subsequently centrifuged at high speed* (40,000 rpm) *for 4.2 hr* through the isokinetic sucrose gradient and the fractions obtained were counted.

1200 atm) for 4.2 hr or at *low speed* (20,000 rpm, average rcf 45,000*g*, rcf at the position of the peak fractions of Post and Pre 51,500*g*, hydrostatic pressure at the same position 300 atm) for 16.8 hr.

Processing of the Gradients after Centrifugation. All gradients were fractionated into about 50 ten-drop fractions. In the experiment in the insert to Figure 3 each fraction was counted directly in 10 ml of Bray's fluid (Bray, 1960). In all other experiments each fraction was diluted with 1 ml of ice-cold buffer A and filtered through a Millipore filter. The filter was washed three times with 2 ml of buffer A, dried, and counted in 5 ml of a toluene-based scintillator. The numbers on the ordinate in the figures (except in the insert to Figure 3) are expressed as per cent of the amount of labeled material in the peak fraction. The minimal number of counts per minute in a peak fraction was 200 and each fraction was counted for at least 20 min.

Results

The curves in Figure 1B reveal that the Pre sediments faster than the Init. The conversion of the slower sedimenting Init to the faster sedimenting form requires the same treatment as the conversion of the Init to the Pre. Thus treatment of the Init with S_1, S_3, GTP, and Ala-tRNA converts it into the faster sedimenting form (Figure 1A). This conversion does not take place however when a wrong AA-tRNA (*i.e.*, Phe-tRNA) is substituted for the correct one, or when tetracycline, an inhibitor of AA-tRNA binding (Suarez and Nathans, 1965; Sarkar and Thach, 1968) is added to the reaction mixture (Figure 1B).

The curves in Figure 2A demonstrate that treatment of the faster sedimenting Pre with S_2 and GTP, which converts it into a Post also converts it into a slower sedimenting form (Figure 2A). In several conditions in which Pre is not converted to Post, the faster sedimenting form is not converted to the slower sedimenting form (Figure 2B); thus, when treated with GTP in the absence of S_2 or with S_2 in the absence of GTP, or if the GTP analog GMPPCP is substituted for GTP, or in the presence of an inhibitor of translocation (either fusidic acid (Tanaka *et al.*, 1969) or thiostrepton (Pestka, 1970)), or if the incubation of the reaction mixture with S_2 and GTP is at 0° instead of 37° (Ono *et al.*, 1969). One of the definitions of a Pre is that its peptidyl residue is not released by puromycin whereas that in a Post is (Traut and Monro, 1964). The curves in Figure 2C demonstrate that indeed most of our Post preparation (over 92%) was reactive with puromycin whereas little of the Pre preparation (less than 20%) was.

The difference in sedimentation velocity (ΔS) between the three complexes centrifuged at high speed was determined by including into an isokinetic sucrose gradient particles of known sedimentation velocity; 30S and 50S ribosomal subunits from *E. coli* and f2 bacteriophage, reported to sediment at 76 S (Zinder, 1968). In these conditions ΔS(Pre·Post) was approximately 4 (Figure 3); ΔS(Pre·Init) was approximately 5; and ΔS(Post·Init) was 1–2 (data not shown).

On the Possible Causes of the Difference in Sedimentation Velocity. The Pre contains one more tRNA than either the Init or the Post. The difference in sedimentation velocity between the complexes ($\Delta S/S > 4/70 > 5\%$) is however too large to be accounted for by the difference in particle weight (Δ particle weight/particle weight $\sim 3 \times 10^4/3.6 \times 10^6 \sim 1\%$) caused by a tRNA molecule. The experiments presented in the subsequent sections on the possible causes of the difference in sedimentation velocities were performed only with the Pre and the Post.

According to Infante and Baierlein (1971) large hydrostatic pressure prevailing during centrifugation at high speed induces dissociation of free ribosomes (*i.e.*, couples of a small and a large subunit not complexed to mRNA and/or tRNA). The dissociation in turn may result in a peculiar sedimentation pattern: The ribosome couples are not always well separated from the subunits, consequently one may deal with an unseparable equilibrium mixture of components dissociating and reassociating during sedimentation. This may give a false impression of the existence of ribosome couples which sediment slower than expected (see also Spirin, 1971). Two of the criteria of such pressure-induced dissociation phenomena are that the apparent sedimentation velocities depend on (a) the hydrostatic pressure and (b) the concentration of ribosomes in the gradient.

Though we were dealing with ribosome·mRNA·tRNA complexes (and pressure induced dissociation was reported not to affect such complexes) we tested them for both of these criteria. (a) We centrifuged one aliquot of a reaction mixture containing both Pre and Post through an isokinetic sucrose gradient at low speed (20,000 rpm) (Figure 4A) and a second aliquot at high speed (40,000 rpm) (Figure 4C). The time of centrifugation at low speed was increased to such an extent that the complex sedimented to the same position in the gradients as in the experiments at high speed. (At this position, the hydrostatic pressure was 1200 atm in high-speed centrifugations, and 300 atm in low-speed centrifugations.) As was done in the case of high-speed centrifugations, we verified that most of the Post (73%) and little of the Pre (12%) was reactive with puromycin after sedimentation at low speed (Figure 4B). Under these conditions no difference was detected between the sedimentation velocities of Pre and Post (Figure 4A).

It could be argued that as a consequence of the longer centrifugation time the extent of diffusion increased and obscured the difference in sedimentation velocities between Pre and Post. However the width of the peaks in the gradients was only 15% greater in the long runs at low speed than in the short ones at high speed.[2] This increase cannot account for the disappearance of the difference in sedimentation velocities. These results reveal that a difference between the sedimentation velocities of Pre and Post is manifested at high-speed centrifugation but not at low-speed centrifugation. (b) To test the effect of ribosome concentration on the sedimentation pattern we applied to sucrose gradients various amounts of Pre and Post. The largest amount was that tested in Figures 2–4. Other aliquots containing one-third, one-sixth, and one-ninth as much were applied (in equal volumes) to other gradients. All of these were centrifuged at high speed in the conditions of Figure 3.

We found that the recovery of each complex in the peak fraction was independent of the amount of complex applied. (Recovery equals the ratio of the amount of complex found in the peak fraction to the amount applied to the gradient.)[3]

[2] The width of the peaks was measured at half their height. The widths were averaged for six runs at high speeds. The average value was 5.2, the maximum 5.5, the minimum 5 for the case of Pre. The average value was 5.2, the maximum 5.5, the minimum 5 for the case of Post. The results of four runs at low speed were also averaged. The average value was 6.0, maximum 7.0, and minimum 5.5, for the single peak of both Pre and Post.

[3] The recovery of the Pre was between 35 and 39%, that of the Post between 29 and 33% in experiments in which the largest amounts of the

Moreover the peaks of Pre and Post sedimented in the same fractions in all cases as in the experiment shown in Figure 3 (data not shown). The width of the peak (plotted as in Figures 1 to 4) was approximately the same in all cases (it varied between 5 and 5.5 mm).

We believe that the independence of the sedimentation pattern of the complexes from their concentration (a) does not support the idea that the difference in sedimentation velocities between Pre and Post is due to a different extent of reversible dissociation of the two complexes, and (b) is consistent with the view that the complexes do not undergo reversible dissociation during centrifugation. Another conceivable interpretation of these results is that there is a complete dissociation of the (slower sedimenting) Post under these conditions, even when the largest amounts of it are tested. This hypothetical dissociation could give rise to 50S subunit·fMet-Ala-tRNA complexes and either free 30S subunits and free f2 RNA or less likely 30S subunit·f2 RNA complexes. If that was the case, the f2 RNA (either free or bound to 30S subunits) should sediment slower than the 50S subunit·fMet-Ala-tRNA complex. The fact that the peak of the f2 RNA (labeled with ^{32}P) sedimented in the same fraction as the peak of fMet-Ala-tRNA (labeled with ^{14}C or ^{3}H in alanine) and the ratio of fMet-Ala-tRNA to f2 RNA in these fractions was the same in the Pre and the Post makes this interpretation unlikely (data not shown).

The results presented so far could be accounted for by one of the following two hypotheses. Large hydrostatic pressure may distort the Pre and the Post to a different extent. The difference in sedimentation velocity manifested during centrifugation at high speed (but not at low speed) may be due entirely or in part either (i) to such a differential distortion (*i.e.*, change in conformation) itself, or (ii) or a (larger) decrease in particle weight of the Post (as compared to the Pre) resulting from the irreversible loss of components triggered by the distortion.

To distinguish between hypothesis i and ii we performed the following experiments. First we centrifuged a mixture of Pre and Post through a sucrose gradient at high speed. This resulted in a partial resolution of the two complexes (Figure 5A). Thereafter we mixed the fractions containing either or both of the two complexes, concentrated the resulting solution, and applied one aliquot to a sucrose gradient, which was centrifuged at low speed, and another aliquot to a sucrose gradient which was centrifuged at high speed.

No difference was detected between the sedimentation patterns of the Pre and the Post in the gradient centrifuged at low speed (Figure 5B). In the gradient centrifuged at high speed the Pre sedimented faster than the Post (data not shown). (In this case there was a slight asymmetry in the sedimentation pattern of the Post probably in consequence of a minor loss of components occurring during the long experiment.) These results may reflect that the centrifugation of the complexes through a sucrose gradient at high speed does not cause an irreversible change in their sedimentation characteristics, *i.e.*, the cause of the difference in sedimentation velocities between the two complexes in these conditions is not the consequence of an irreversible loss of components.

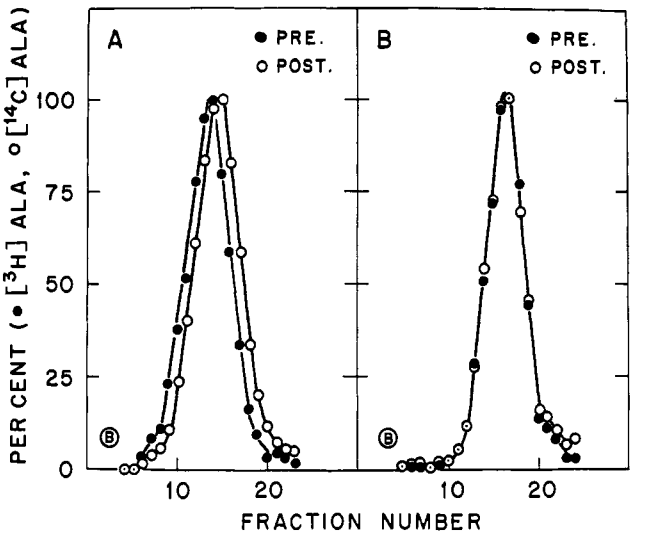

FIGURE 5: There is no detectable difference between the sedimentation velocities of Pre and Post centrifuged through a sucrose gradient at low speed even if the mixture of the two complexes analyzed is one which had been partially resolved earlier by centrifugation through a sucrose gradient at high speed. [^{3}H]Ala-labeled Pre and [^{14}C]Ala-labeled Post (prepared as described in the Experimental Section) were mixed. Aliquots (0.7 ml) of the mixture were applied to each of four isokinetic sucrose gradients. These were centrifuged at high speed (40,000 rpm) for 4.2 hr and collected in fractions. The fractions from one gradient were counted (A). The fractions containing most of the radioactivity (fractions 12–18) from the other three gradients were pooled (total volume ~ 6 ml) and the resulting solution was concentrated (final volume ~ 1 ml) by adding dry Sephadex G-25 (course) and dialyzed against buffer A. The solution obtained was applied to an isokinetic sucrose gradient which was centrifuged at low speed (20,000 rpm) for 16.8 hr. The fractions were collected and counted (B).

Consequently, our findings are inconsistent with hypothesis (ii) and are in line with hypothesis (i).

It should be noted that the recovery of each complex in the 70S region of the sucrose gradient was lower in the case of high-speed centrifugation than in the case of low-speed centrifugation (see footnote 3). This indicates that the dissociation of each complex was increased by the large hydrostatic pressure. However, we determined the positions of the complexes in the gradients by counting that portion of the radioactivity in each fraction which was retained by Millipore filters, *i.e.*, fMet-Ala-tRNA bound to ribosomes. The plot of these data gave symmetrical curves. This consideration, taken together with the lack of effect of ribosome concentration on the sedimentation pattern and with the lack of an irreversible effect of large hydrostatic pressure on the sedimentation pattern of the mixture of Pre and Post, supports the validity of our hypothesis.

Discussion

Our studies involving high- (and low-) speed centrifugations of ribosome complexes through sucrose gradients lead to the following results. (i) As tested in a gradient centrifuged at high speed, the conversion of the slower sedimenting Init to the more rapidly sedimenting complex was found to require the same treatment as its conversion to a Pre, and the conversion of the more rapidly sedimenting Pre to the more slowly sedimenting complex was found to require the same treatment as its conversion to the Post. (ii) The sedimenta-

complexes were tested; and 38 and 30%, respectively in those in which one-ninth as much was tested. All these experiments involved centrifugation at high speeds. The recoveries were higher, between 75 and 80% for both Pre and Post, in the experiments in which only low-speed centrifugations were performed.

tion patterns (including the per cent recovery in the peak fractions) of Pre and Post (centrifuged at high speed) were apparently not altered when the concentration of the ribosome complexes in the gradients was decreased ninefold. (iii) No difference was detected between the sedimentation velocities of Pre and Post centrifuged through a sucrose gradient at low speed. (iv) No difference was detected between the sedimentation velocities of Pre and Post centrifuged through a sucrose gradient at low speed even if the mixture of the two complexes analyzed was one which had been partially resolved earlier by centrifugation through a sucrose gradient at high speed.

We believe that result ii makes it improbable that the difference in sedimentation velocities between the Pre and the Post should be due to a difference in the pressure-induced reversible dissociation of the two complexes into subunits. Actually Infante and Baierlein (1971) who discovered the pressure effect on free ribosomes, also noted that complexed ribosomes may not be dissociated by pressure.

We propose that our results may be explained by a differential distortion (*i.e.*, change in conformation) of the two complexes caused by the large hydrostatic pressure prevailing during centrifugation at high speed. The fact that large hydrostatic pressure blocks protein synthesis *in vivo* (Landau, 1967) as well as *in vitro* causing a decrease in the stability of the AA-tRNA·ribosome·mRNA complex in the cell-free system (Arnold and Albright, 1971) is in line with this hypothesis.

Changes in ribosome conformation in the course of the conversion of the pretranslocation complex to the posttranslocation complex have been predicted (Nishizuka and Lipmann, 1966; Spirin, 1969). Indirect support for such a change was provided by finding differences in the rate of tritium exchange between the two ribosome complexes (Chuang *et al.*, 1971). Our results are in line with those of Spirin (1971) and Infante and Baierlein (1971) indicating that a difference in sedimentation velocity between Pre and Post in sucrose gradients centrifuged at high speed and large hydrostatic pressure is not a sufficient proof for a difference in conformation between the complexes.

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