BINDING OF AMINO ACYL-sRNA TO RAT LIVER POLYSOMES

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Received July 31, 1963

INTRODUCTION

Soluble RNA has long been known to be transiently bound to ribosomes during protein synthesis (Hoagland et al., 1960; Takanami, 1962a, b). Since evidence is accumulating that protein synthesis is effected by aggregates of several ribosomal units (polysomes or ergosomes), complexed by strands of messenger RNA (Risebrough et al., 1962; Warner et al., 1962; Wettstein et al., 1963; Staehelin et al., 1963; Noll et al., 1963; Penmann et al., 1963), it seemed worthwhile to investigate the distribution of amino-acyl-sRNA on these active ribosomal aggregates during protein synthesis in vitro.

Preliminary evidence is presented here indicating that amino-acyl-sRNA is initially bound to actively incorporating heavier aggregates and remains attached to the monomeric units upon the progressive disruption of the polysome structure that parallels protein synthesis in vitro.

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MATERIALS AND METHODS

Livers from male albino rats were homogenized in Medium A (Robinson and Novelli, 1962) containing 5 X 10^{-3} M mercaptoethanol; the homogenate was centrifuged at 30,000 X g to yield a postmitochondrial supernatant, to which sodium deoxycholate was added (1.2% final concentration). Ribosomes were prepared by further centrifugation at 105,000 X g for 90 minutes through two layers of sucrose of different density according to Wettstein (1963). The bottom sucrose layer was 1.0 M.

Ribosomes were incubated under conditions of protein synthesis as indicated under Fig. 2. At selected periods of time during incubation, samples were taken and analyzed by zonal centrifugation in a sucrose density gradient (SDG) according to Britten and Roberts (1960). Each fraction was tested for radioactivity bound to protein, hot trichloroacetic acid (TCA) insoluble, and for radioactivity bound to sRNA, insoluble in cold TCA but extractable with hot TCA. For this purpose, the technique developed by Bollum (1959) was used as modified by Mans and Novelli (1961). Radioactivity bound as amino-acyl-sRNA was equated to the difference between the value of the cold TCA insoluble radioactivity and the radioactivity remaining after hot TCA extraction. The values obtained by this procedure were found to agree closely with those obtained by splitting the amino acid from sRNA with Na_2CO_3—$NaHCO_3$ buffer, pH 10, or by RNase digestion of the incubated ribosomes.

The approximate sedimentation rates of ribosomal aggregates resolved in the SDG were estimated by comparison with particles of known sedimentation rate centrifuged under identical conditions. Initial calibration was performed by analytical ultracentrifugation, with washed ribosomes prepared according to Korner (1961). The number of repeating ribosomal units in aggregates of various size is tentative and has been used here for descriptive purposes.

RESULTS AND DISCUSSION

The SDG pattern of freshly prepared ribosomes, represented in Fig. 1, clearly shows that the majority of the ribosomes sediment in a largely unresolved region covering aggregates of sedimentation rate ranging from 96 S to approximately 200 S whereas the monomeric 67 S unit includes only a small percentage of the area under the optical density curve.

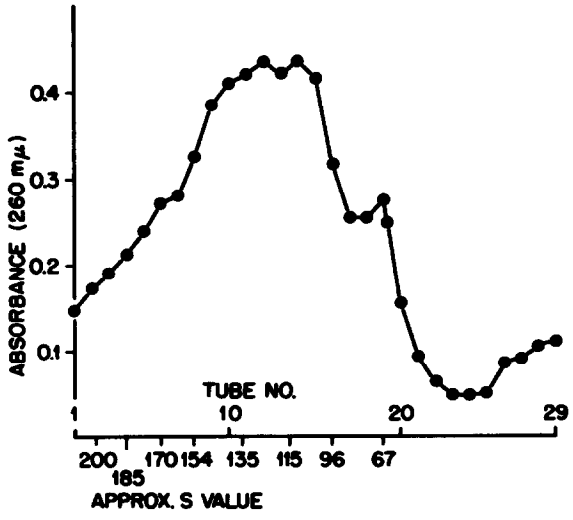

Fig. 1. Sedimentation in a sucrose gradient of freshly prepared rat liver ribosomes, 1 ml of the ribosomal suspension in medium A (2 mg of ribosomal protein) was layered on top of a 25-ml sucrose gradient (exponential 10-34%) containing 0.01 M Tris, 0.004 M succinic acid, and 0.005 M $MgAc_2$, pH 7.6, in a Spinco SW 25 swinging-bucket tube. The gradient solution was centrifuged 2 hours at 25,000 rpm. The relative positions of aggregates of various sizes has been determined according to Martin and Ames (1961), by assuming the position of the 67 S as marker.

In the experiments reported in Fig. 2, rat liver ribosomes were incubated under conditions of protein synthesis with C^{14}-leucine for the indicated periods of time and analyzed by SDG to determine the distribution of the ribosome-bound C^{14}-amino-acyl-sRNA with respect to aggregate size.

For comparison, the distribution of the labeled nascent protein is reported in Fig. 3.

Visual inspection of the optical density distribution at 5 and 15 minutes respectively of incubation (Fig. 2a and b) reveals a progressive increase of the relative proportion of the 67 S and 96 S peak along with parallel decrease of the amount of aggregates of heavier size. After 30 minutes of incubation (Fig. 2c), most of the ribosomal population is present in the 67 S form. Centrifugation of the ribosomes incubated for 30 minutes in a SDG prepared in the absence of magnesium results in further accumulation of the monomer pool (Fig. 2d).

After 5 minutes of incubation, the radioactivity of the ribosome-bound amino-acyl-sRNA (Fig. 2a) is distributed in peaks coincident with the positions predictable for particles of size covering the range from 67 S to approximately 200 S. The greatest amount is in association with aggregates of sedimentation coefficient greater than 96 S, with a major peak corresponding to aggregates of sedimentation constant 135 and 154 S; only a small amount of radioactivity is found associated with the monomer pool, whose specific activity is the lowest one. After 15 minutes of incubation (Fig. 2b), there is a

relative reduction in the radioactivity associated with heavy aggregates, paralleled by a corresponding increase of the radioactivity included in the region of aggregates of sedimentation constant of 135. An increase of radioactivity in the region of the monomer pool is also observable. After 30 minutes of incubation (Fig. 2c), the major peak of radioactivity is associated with the monomers, followed by the dimer and the trimer

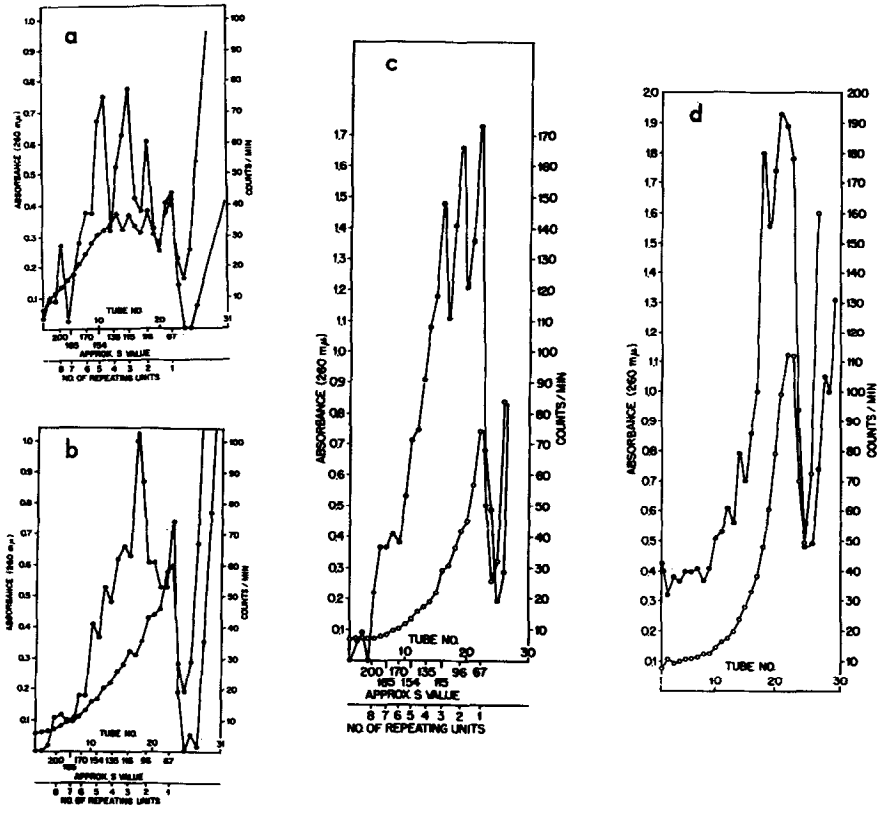

Fig. 2. Distribution of C^{14}-amino-acyl-sRNA in a SDG of incubated ribosomes. Open symbols show the optical densities at 260 mμ. Solid symbols show counts per minute. Incubation was carried out at 37°C in a mixture containing (per ml): Tris buffer (pH 7.6), 25 μmoles; $MgAc_2$, 4 μmoles; KCl, 50 μmoles; beta-mercaptoethanol, 20 μmoles; creatine phosphate, 15 μmoles; creatine kinase, 10 μg; ATP, 4 μmole; GTP, 1 μmole; L-leucine U-C^{14} (SA 246 μc/μmole), 0.5 μc; 1 mg ribosomal protein; and 4 mg of protein of postmicrosomal supernatant. Aliquots (1 ml) of the incubation mixture were centrifuged in SDG under the conditions reported in Fig. 1 at, respectively, 5 minutes (A), 15 minutes (B), and 30 minutes (C). An additional sample taken at 30 minutes was centrifuged in SDG prepared in the absence of Mg^{++} (D). Eleven-drop samples were collected; 0.2 ml of each was plated on filter paper disks and processed for radioactivity measurements. The remainder, diluted with 2.5 ml of water, was used to measure the absorption at 260 mμ. Counts associated with amino-acyl-sRNA were calculated as described in Methods. Aggregate size in repeating 67 S units has been tentatively calculated as described elsewhere (Mandelkern et al., 1951; Gierer, 1963).

peaks, respectively, while a sharp fall in the relative amount of radioactivity is observed in the area of the heavy aggregates. Centrifugation of the ribosomes incubated for 30 minutes, in a sucrose gradient prepared in the absence of magnesium (Fig. 2d) results in further shifting of the amino-acyl-sRNA, most of the radioactivity becoming associated with the monomeric units and a minor amount with the dimer and trimer peaks.

The results of a parallel determination of the distribution of the labeled nascent protein in aggregates of various size are given in Fig. 3. The radioactivity is again mainly associated with the heavy aggregates after 5 minutes of incubation, and then undergoes a progressive shifting towards the region of light aggregates, along with the progressive monomerization of polysomes, leading to predominant accumulation of labeled monomers.

This latter result confirms the finding of Noll et al. (1963) supporting the view that protein synthesis proceeds by stepwise splitting from the polysome of individual particles, each bearing a nascent peptide chain.

Taken as a whole, our findings indicate that, under conditions of protein synthesis, the amino-acyl-sRNA is largely bound to polysomes and follows their progressive monomerization during amino acid incorporation. This fact strongly suggests that the 67 S

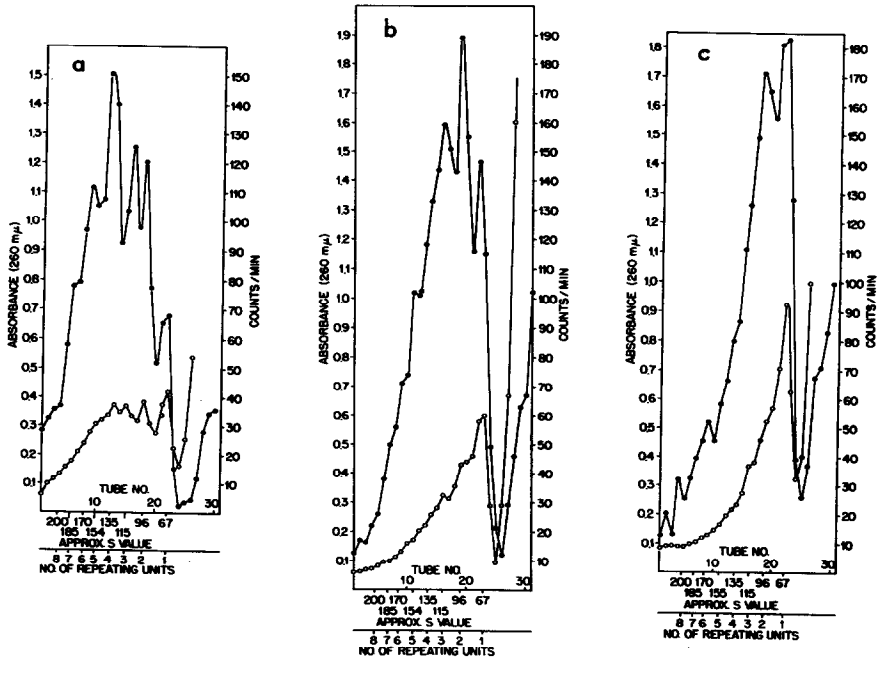

Fig. 3. Distribution of labeled protein in a SDG of incubated ribosomes. Open symbols show the optical densities at 260 mμ. Solid symbols show counts per minute. Same experiment as in Fig. 2.

units are the site of binding of amino-acyl-sRNA to polysomes, although partial attachment to the inter-ribosomal RNA cannot be excluded at present. The linkage between amino-acyl-sRNA and ribosomes appears to be rather a stable one, being resistant to sucrose gradient centrifugation even in the absence of magnesium.

Experiments are in progress aimed at ascertaining whether amino-acyl-sRNA is attached to the 50 S or to the 30 S components of the 67 S particle. Also being investigated is whether the radioactive amino-acyl-sRNA associated with the 67 S peak originates entirely from monomerization of heavy aggregates or whether the monomeric units are also able to bind amino-acyl-sRNA. It is already apparent, however, from comparing specific activities of different classes of ribosomes after 5 minutes of incubation, that the monomeric units should be far less able than polysomes to effect this binding.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Dr. Peter Pfuderer for performing ultracentrifugation analyses and for helpful discussions during the course of this investigation.

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