CYTOPLASMIC PARTICLES AND AMINOACYL TRANSFERASE I ACTIVITY

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ABSTRACT

It has been possible to show by electron microscopy of samples selected from sucrose gradients that particles of specific size and shape are present in supernatant fluids derived from nucleated animal and plant cells, but not in extracts from *Escherichia coli*. Aminoacyl transferase I activity in these same gradients sediments in two peaks representing material of approximately 5–7S and 18–20S. A rectangular particle, 100 × 145 A in size, sediments at 19S and coincides with the second peak of transferase I activity. The possibility that the rectangular particle may be a "carrier" particle associated with transferase I is discussed.

During the course of studies on the fine structure of ribosomes, it was observed that smaller particles with well defined structure sedimented in sucrose gradients together with the 45S ribosomal subunit. In an endeavor to ascribe a function to the particles, it was found that the enzyme aminoacyl transferase I had a peak of activity that coincided with a region of the sucrose gradient in which certain of the particles were concentrated. Since transferase I has been shown to catalyze the binding of aminoacyl transfer RNA to ribosomes in the process of peptide chain elongation (7), experiments were designed to examine further the apparent relationship between particles and transferase I activity. This report will describe the structure and occurrence of these particles in various cell types. It will be seen that transferase I activity in freshly prepared cytoplasmic extracts is associated with particulate material sedimenting at 19S. The association is unstable and its physiological significance cannot be assessed at the present time.

MATERIALS AND METHODS

Supernatant Fluids

Supernatant fluids were prepared from the following tissues and examined for the presence of particles after sedimentation through sucrose gradients.

PLASMA-CELL TUMORS MOPC-104E AND RPC-20 (TRANSPLANTED IN BALB/C MICE) AND NORMAL BALB/C MOUSE LIVER: 25% homogenates of these tissues were prepared in 0.25 M sucrose buffered with 0.05 M Tris-Cl, pH 7.6, 0.025 M KCl, 0.005 M $MgCl_2$ (Medium A). The homogenates were centrifuged for 10 min at 10,000 *g* to derive the supernatant fluid.

RABBIT RETICULOCYTES: Phenylhydrazine-HCl-induced rabbit reticulocytes were lysed in 0.0015 M $MgCl_2$ (2 volumes per volume of packed cells) and mixed with 4 volumes of 0.01 M Tris-Cl, 0.01 M KCl, 0.0015 M $MgCl_2$, pH 7.4 (10). The supernatant fluids were derived from a 10-min centrifugation at 10,000 *g*.

HUMAN ERYTHROCYTES: The serum and buffy coat were removed from freshly drawn blood and the cells were washed twice with 0.14 M NaCl, 0.005 M KCl, 0.0015 M $MgCl_2$. 4 ml of packed cells were

lysed and centrifuged by the procedure used for reticulocytes.

PEA SEEDLINGS: Seeds of *Pisum sativum* were sprouted and grown in the dark for 7 days. The 4- to 6-in. long stems and cotyledons (1 g) were chopped and then ground in a mortar with sand soaked with Medium A. The slurry was filtered through cheesecloth to yield about 6 ml of yellow liquid which was centrifuged for 10 min at 10,000 *g* for preparing the supernatant fluid.

ESCHERICHIA COLI: Supernatant fluids were derived either by (*a*) rupturing log phase cells in a French pressure cell at 13,000 psi and centrifuging the slurry for 30 min at 30,000 *g* in 0.05 M Tris-acetate, pH 7.2, 0.01 M mg acetate, 0.05 M NH_4Cl and 10^{-4} M dithiothreitol (DTT) or by (*b*) lysing spheroplasts by freezing and thawing, hand homogenizing in a glass tube with a Teflon pestle, and centrifuging at 10,000 *g* in 9% glycerol, 0.05 M Tris-Cl, pH 7.4, and 0.001 M $MgCl_2$ (9).

AMINOACYL TRANSFERASES I AND II: Partially purified transfer factors were prepared and resolved on Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) essentially as described by Gasior and Moldave (1). The bulk of transferase I was excluded from Sephadex G-200. This material was used for sucrose density gradient analysis. Fractions containing transferase II were pooled and used in the assays for transferase I.

RIBOSOMES: Rat liver ribosomes were prepared by the method of Wettstein et al. (11). The pellets were resuspended and centrifuged three times through a discontinuous sucrose gradient containing 0.5 M NH_4Cl (6).

ASSAY FOR AMINOACYL TRANSFERASE I: Transferase I activity was measured in the presence of an excess of transferase II by the transfer of amino acids from aminoacyl-tRNA into protein. The reaction mixture (total volume 0.5 ml) contained, in addition to the fraction to be assayed: 25 μmoles of Tris-HCl, pH 7.4, 3 μmoles of $MgCl_2$, 40 μmoles of NH_4Cl, 30 μmoles of 2-mercaptoethanol, 0.18 μmoles of guanidine triphosphate (GTP), ribosomes (75–150 μg of protein), transferase II (100–200 μg of protein), and 7–10 μg of leucyl-^{14}C-tRNA (360 mμCi/mg). *E. coli* aminoacyl-tRNA (^{12}C UL) was obtained from New England Nuclear Corporation.

GRADIENTS: 2 ml of the various supernatant fluids and the transferase I preparation were layered over 28 ml of 15–30% (w/w) linear sucrose gradients and centrifuged 19 hr at 25,000 rpm and 5°C in a SW 25.1 rotor of a Spinco Model L2-65 ultracentrifuge. The gradient solutions contained one of the following buffers: (*a*) 6.25 mM Tris-HCl, pH 7.4, and 0.5 mM $MgCl_2$; (*b*) 10 mM Tris-HCl, pH 7.5, 10 mM KCl, and 1.5 mM $MgCl_2$; (*c*) 2 mM potassium phosphate, pH 7.4, and 1.5 mM $MgCl_2$. 1-ml samples were collected from the top of the tube while UV absorption was monitored at two wavelengths. Gradients and samples were kept at 0–5°C.

ELECTRON MICROSCOPY: Droplets of fluid from the gradient tubes were negatively stained in 2% potassium phosphotungstate, pH 6.2, or 2% uranyl acetate, pH 4.2. Electron micrographs were taken at an initial magnification of 60,000 with a Siemens Elmiskop 1A with a double condenser, 50 μ objective aperture, and 80 kv accelerating voltage.

RESULTS

Morphology and Distribution of Particles in Various Tissues as Related to Sedimentation Velocity

When fluid from individual tubes from the sucrose gradients was stained and examined in the electron microscope, several different particles with distinctive morphology were observed to have sedimented between 18 and 32S. Particles of this nature were found in extracts of all the nucleated cells examined, but in spite of an intensive search, it was not possible to demonstrate their presence in supernatant fluids obtained from *E. coli*. The morphology and distribution of particles were studied most extensively in the supernatant fluids from the two plasma cell tumors, MOPC 104E and RPC 20. The optical density (OD) tracing at E_{260} and E_{280} obtained by monitoring the sucrose density gradient after centrifugation of these fluids was characteristic for the tumors and was completely reproducible. In a typical tracing shown in Fig. 1, most of the absorbing material is concentrated in a region encompassed by tubes 3–9. The OD falls rapidly from a peak at tube 5 to a distinct shoulder lying between tubes 10 and 12 (18–20S), and rises in a broad, low peak between tubes 14–17 (30–32S) before the region of the 45S ribosomal subunit appears between tubes 20 and 25. The 60S ribosomal subunit peak is compressed at the bottom of the gradient tube while the 80S ribosomes and polysomes are completely pelleted.

When the gradient was sampled from the top for electron microscopy, a rectangular particle, first seen in tube 9, was found to be concentrated at the OD peak in tubes 11–12 (Fig. 2 *a–e*). Together with it in this fraction were small spherical or ring-shaped particles (Fig. 2 *b*, *e*). At tube 12, a particle shaped like an irregular disk could first be seen (Fig. 3 *a*, arrows). It was more concentrated in tubes 12 and 13 than the rectangular particles. In the broad OD peak between tubes

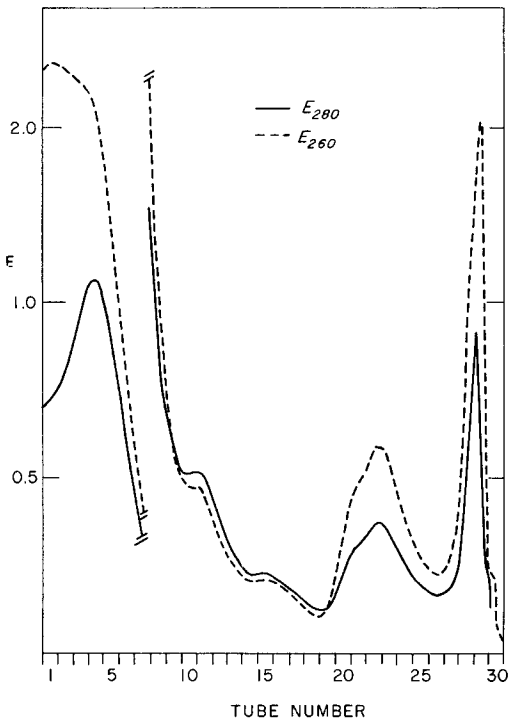

FIGURE 1 Sucrose density gradient pattern typical of supernatant fluids derived from the plasma-cell tumor, MOPC 104E. The height of the initial portion of the curve has been reduced five times; tube 1 represents the top of the gradient. The shoulder at tubes 11–12 coincides with the concentration of rectangular particles (see Fig. 2 *a–e*). The low peak at tubes 14–17 coincides with the appearance of rectangular particles joined with a particle shaped like an irregular disk (see Fig. 3 *a–e*). The peak at tubes 20–25 coincides with the 45S ribosomal subunit.

14 and 17, the rectangular particles were again concentrated, but instead of being free as they were in the first peak, in most instances they were now attached to another particle which sometimes appeared to be identical with the irregular disk (Fig. 3 *a–e*).

The rectangular particles measured 100–145 A on the average (Fig. 2 *a–e*). They appeared to be made up of at least three units, two of which formed the ends of the particle and could be seen as a line 100 A long by 30 A wide. The area lying between the two ends was ill defined, but occasionally could be resolved in the shape of a ring (Fig. 2 *c* and *d*). The small spherical or ring-shaped particles were about 80 A in diameter and were very similar in shape and dimension to the center portion of the rectangular particles (Fig. 2 *b* and *e*). The irregularly shaped disk averaged 145 A in diameter and, in some aspects, had a hole or depression in the center (Fig. 3 *a*, arrows). While the particles attached to the rectangles appeared in some cases to be morphologically identical to the 145 A disks, in others they assumed a trefoil shape or some less clearly delineated form (Fig. 3 *a–e*).

The particles are probably protein in composition. The ratio of E_{260} to E_{280} associated with the regions in which the particles were concentrated was more typical of protein than nucleoprotein (Fig. 1). They resisted digestion with deoxyribonuclease, ribonuclease, phospholipase A, B, and C, but were sensitive to digestion with pronase and deteriorated when incubated at 37°C in Tris-Cl buffer. In addition, no evidence could be obtained for rapid labeling of RNA in the particles, using uridine-^{3}H as a precursor.

Aminoacyl Transferase I Activity in Sucrose Gradients of Supernatant Fluids from Plasma Cell Tumors and Rat Liver

When gradients of plasma cell tumor or rat liver extracts were assayed for transferase I activity, two peaks of activity could be demonstrated repeatedly (Fig. 4). The first peak fell between tubes 4 and 6 and corresponded to material in the neighborhood of 5–7S. The second peak fell in tubes 10–12 and coincided with the tubes in which the rectangular particles were most heavily concentrated. In assaying for the enzyme, the increase in activity relative to increased amounts of added fraction from either tube 5 or 10 was similar, and the activity resulting from a mixture of equal aliquots from these two peak tubes was additive, suggesting that the enzyme in the two peaks was the same.

The presence of two peaks of activity was in accord with the findings of Richter and Klink (4) and Schnier and Moldave (5) that transferase I existed in more than one form. It was of interest, therefore, to test whether the enzyme sedimenting at 18–20S represented a stable complex which could withstand sedimentation through a second gradient. Tubes 10 and 11 from the gradient shown in Fig. 4 were dialyzed against buffer for removing the sucrose and were resedimented on gradients in the usual way. Over half of the enzyme activity was lost in this maneuver but, instead of one peak, two peaks of activity corre-

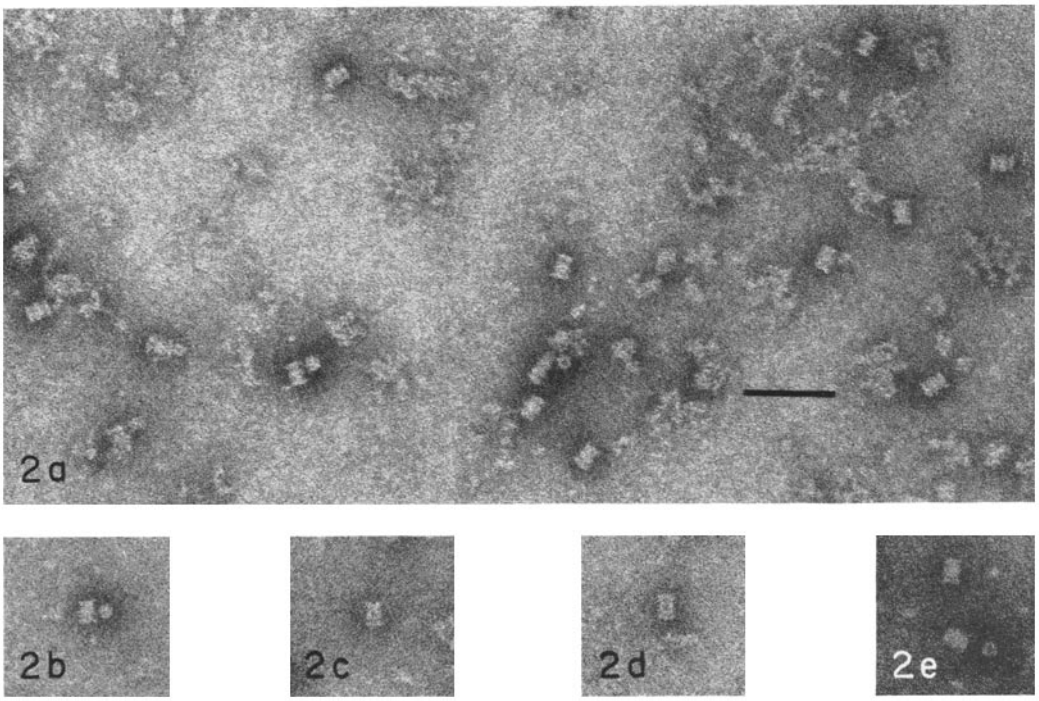

FIGURE 2 *a* Representative over-view of rectangular particles and amorphous material present in a 15-times dilution of fluid sampled from tube 10 derived from the sucrose gradient. *b*, Spherical particle, 80 A in diameter, beside a rectangular particle. *c* and *d*, Rectangular particles in which the central portion appears as a 80 A ring. *e*, Two rectangular particles and a ring-shaped particle which is similar in dimensions to the central portion of the particles in Fig. 2 *c* and *d*. × 240,000. Line represents 500 A.

sponding to two peaks of optical density were obtained and the rectangular particles still sedimented in tubes 10–12, although their number was greatly reduced.

Purified Aminoacyl Transferase I

The gradient pattern obtained with purified transferase I showed a major OD peak in tubes 5–7 and a smaller one in 11–12. All of the enzyme activity was concentrated in the peak at tubes 5–6. While no transferase I activity was detected in the smaller peak, it was found to contain very few rectangular particles (Fig. 6) and to consist primarily of the α_2 macroglobulin (8).

Numerous particles, 50–100 A in size, were concentrated in tubes 5–6 (Fig. 5 *a*, *b*), and particles of similar appearance but in diminishing concentration were seen in tubes 3–4 and 7–8. The enzyme is probably represented by these particles, but attempts to establish a convincing structure for it were frustrated by the technical difficulty of the negative staining. Staining with either potassium phosphotungstate or uranyl acetate failed to produce images that permitted definitive interpretation of the ultrastructure of the molecule.

DISCUSSION

By electron microscopy of samples selected from sucrose density gradients, it has been possible to show that a group of particles of specific size and shape were present in supernatant fluids derived from nucleated plant and animal cells but not in extracts from *Escherichia coli*. Furthermore, when extracts from the cells of mouse and rat liver and two mouse plasma-cell tumors were repeatedly studied it was found that only five types of particles could be demonstrated to sediment between 18 and 32S. These were a 100 × 145 A rectangular particle, two 80 A particles of such size and shape that they could be fragments of the rectangular particles, a 145 A disk-shaped particle, and a particle resembling α_2 macroglobulin. A more

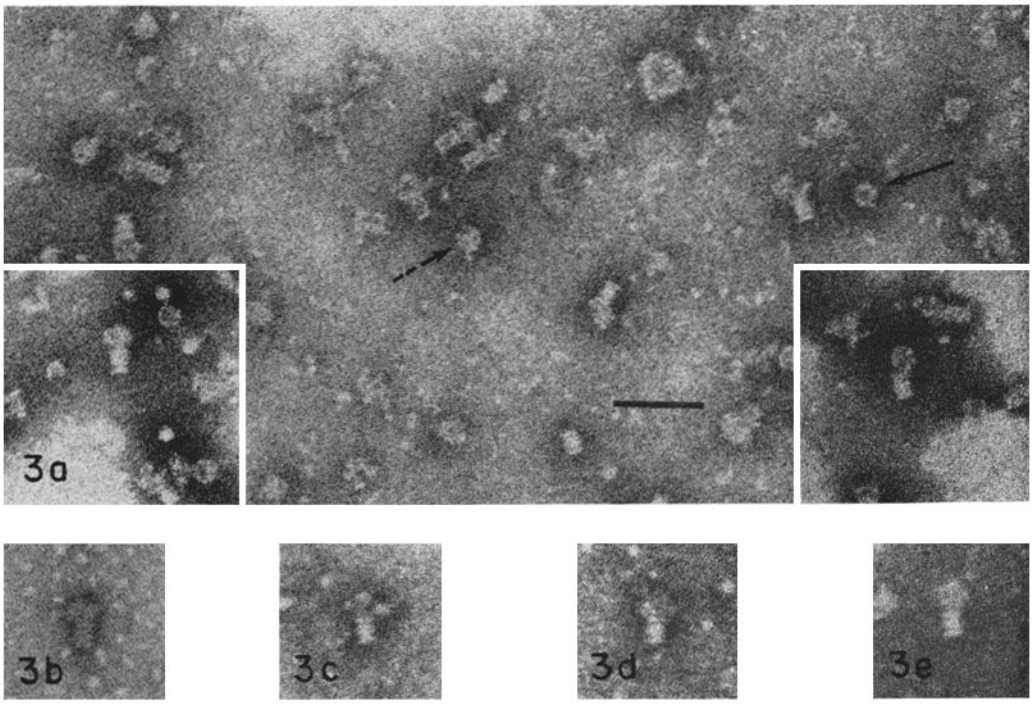

FIGURE 3 *a* Representative over-view of particles in fluid from tube 15 diluted five times. Most of the rectangular particles are associated with irregularly shaped disklike particles (arrows). MOPC 104E plasma-cell tumor. *b*, Particle derived from RPC-20 plasma cell tumor. *c* and *d*, Particles derived from pea seedlings. *e*, Particle derived from human erythrocytes. Potassium phosphotungstate stain. × 240,000. Line represents 500 A.

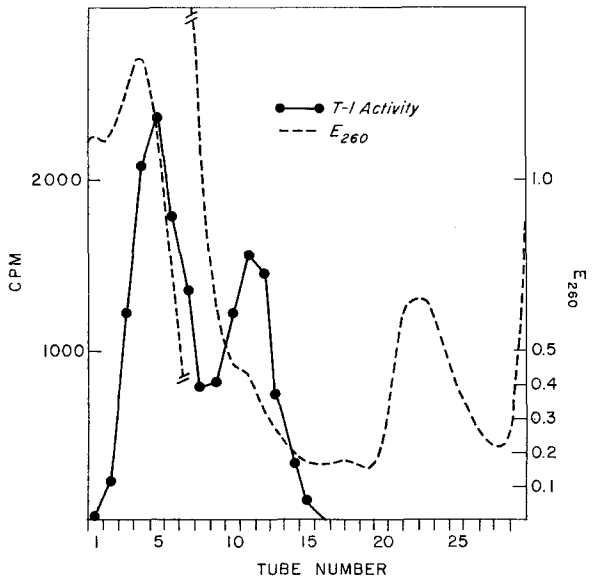

FIGURE 4 Curves showing the relationship between optical density and transferase I activity. RPC-20 plasma cell tumor. Aliquots (10 μl) were assayed using leucyl-^{14}C-tRNA.

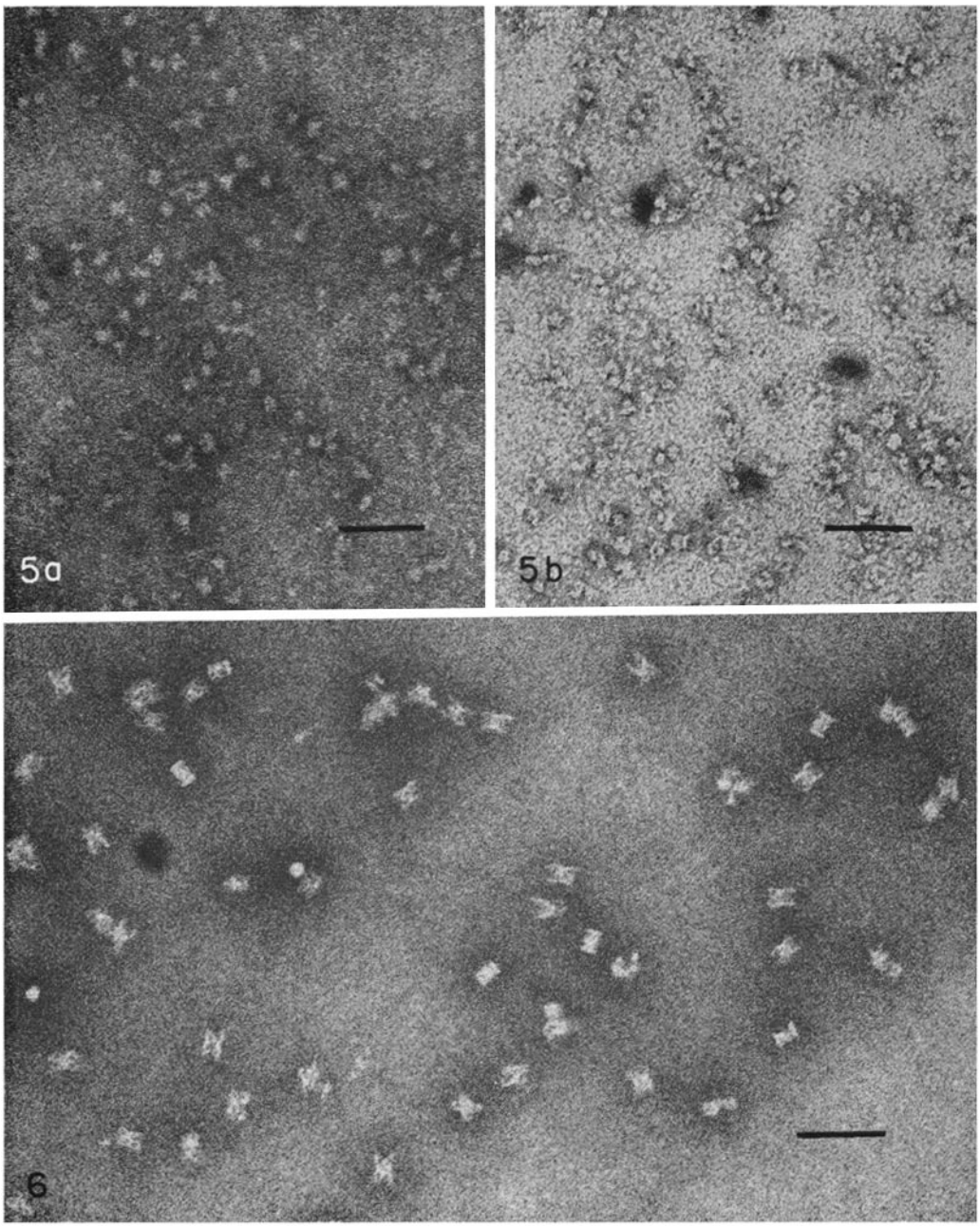

FIGURE 5 *a* and *b* Particles from gradient tube containing peak of purified rat liver transferase I activity. Although the particles are fairly uniform in size, no convincing detailed ultrastructure can be deduced from these photographs. *a*, Potassium phosphotungstate. *b*, Uranyl acetate. × 240,000. Line represents 500 A.

FIGURE 6 Particles derived from tube 11 of a sucrose gradient of purified rat liver transferase I. Over 50% of the particles in this micrograph resembled α_2 macroglobulin, while rectangular particles comprised only 11% of the total. Potassium phosphotungstate. × 240,000. Line represents 500 A.

complex structure resulted from the apparent joining of the 145 A disk to the rectangular particle. Its significance is unknown. In addition to these well defined particles, a quantity of amorphous material was always present in the negatively stained preparations sampled from the gradient. We have reason to believe that much of the amorphous material represents denatured and aggregated protein since it increased in size and bulk with loss of integrity of recognizable structures and enzyme activity.

Structures very similar in morphology, but different in size from the 100 × 145 A rectangles and the 80 A rings have been reported by Harris (2) who was able to release quantities of 110 × 180 A rectangular and 110 A ring-shaped particles from human and ox erythrocyte ghosts by prolonged dialysis against 0.01 M phosphate buffer or by treatment with sodium dodecyl sulfate. The structure of the rectangular particle was interpreted as a cylinder composed of a stack of four ring-shaped particles. They were believed to be macromolecular components of the erythrocyte membrane. Whether or not they are identical to the rectangular particles that we have observed is uncertain. In our hands, the particles do not withstand dialysis and do not appear to be membrane bound.

A suggestion for a function related to one of these particles comes from the experiments with transferase I reported here. The enzyme in tissue extracts appears to exist in two molecular forms, one sedimenting in sucrose density gradients at 6S and the other at 18–20S. The coincidence of the rectangular particle with enzyme activity sedimenting at 18–20S tempts one to associate the enzyme function with the particle. Aside from cosedimentation in sucrose gradients, there is some additional evidence for this proposed association. The rectangular particles are not present in bacteria, and transferase I catalyzes the binding of aminoacyl transfer RNA to ribosomes in yeast and metazooan systems (4, 7), but not in bacteria (3). Schnier and Moldave (5) found, as we have, that the different forms of transferase I had additive rather than synergistic effects in the enzyme assay system. Further, they have demonstrated the existence of three separate molecular weight species of transferase I and have found that the highest molecular weight species was readily converted to an intermediate one. They suggested that transferase I activity could be bound to a larger particle not involved in protein synthesis. These observations have their counterpart in our results with the regradient of the rectangular particles (tubes 10 and 11) which showed that a slower sedimenting, enzymatically active species was derived from a larger one.

The rectangular particle possibly represents the "carrier" molecule, suggested by Schnier and Moldave, to which the smaller enzyme molecule is attached. Both the morphological integrity of the particles and the enzyme activity are destroyed and reduced by the gentlest of manipulations, and only when both of them can be stabilized and purified can a definitive assessment of their relationship to each other be made.

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A brief description of the particles discussed in this article has appeared in *J. Cell Biol.* 1968. **39**:123 *a*. (Abstr.)

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