Berger, E.M. State University of New York, Albany, N.Y. Isolation and dissociation of Drosophila ribosomes.

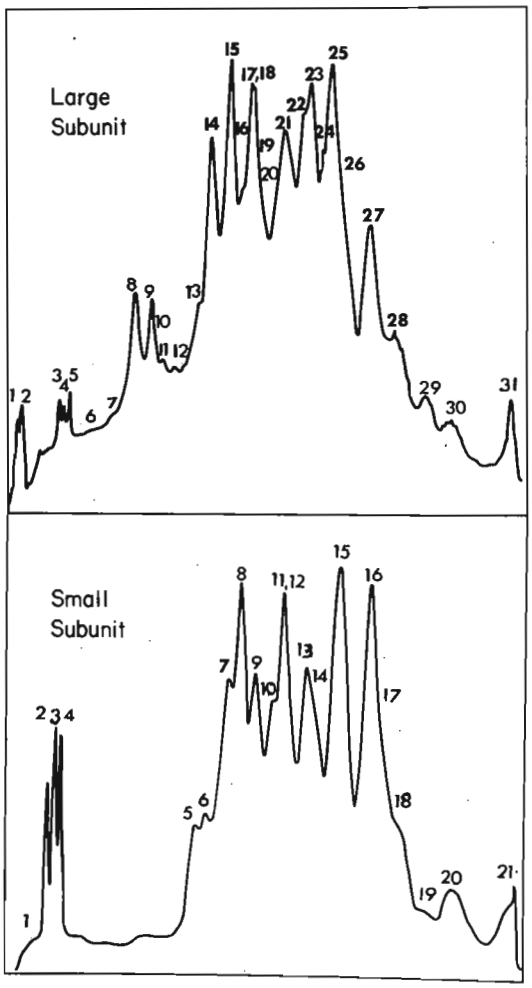

Figure 1. Densitometric scan of 40 S and 60S ribosomal proteins following SDS acrylamide gel electrophoresis. Migration is toward the right.

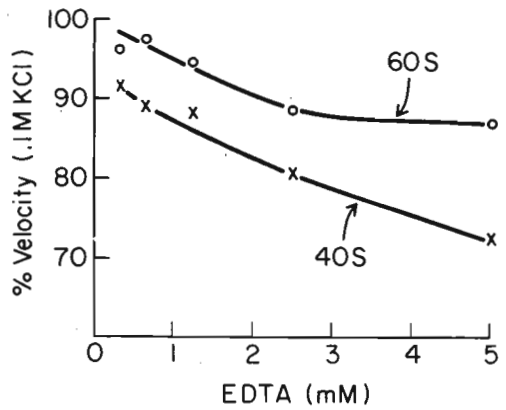

Figure 2. Reduction of ribosomal subunit sedimentation velocity following dissociation in varying concentrations of EDTA. Percent velocity is with respect to subunits dissociated in the absence of EDTA and sedimented in parallel gradient tubes.

Drosophila virilis ribosomes isolated from adult homogenates (1) were found to have a sedimentation coefficient in sucrose gradients of about 80S; with respect to parallel standards including E. coli monosomes, and monosomal subunits. Drosophila ribosomes were completely dissociated after incubation in 0.01 M Tris, pH 7.3, 0.1 M KCL into subunits having S_{20} coefficients of 40S and 60S. Proteins isolated from small and large subunits by the acetic acid method (2) were analyzed in 10% acrylamide, 0.1% SDS gels (3). In the small subunit 21 bands of staining were observed, while large subunits yielded 31 bands (Figure 1a and b).

One interesting effect on ribosome dissociation was noted when Na_2 EDTA, a chelating agent, was included in the monosome dissociating buffer. With increasing EDTA concentration, the apparent sedimentation velocity of both subunits decreased (Figure 2). This reduction appears to involve a conformational change in the subunit structure induced by cation (M_g++ probably) removal since:

(1) The 260/280 ratios of the altered subunits are not affected.

(2) The protein spectra of altered subunits are indistinguishable from the OmM EDTA controls.

(3) The alteration can be reversed by adding M_g++ back to isolated subunits, and resedimenting them in sucrose, including in parallel tubes subunits dissociated in the absence of EDTA.

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References: 1. Lambertson, A., S. Rasmuson and G. Bloom 1970, Molec. Gen. Genetics 108:349; 2. Leboy, P., E. Cox and J.G. Flaks 1964, PNAS, U.S. 52:1367; 3. Weber, K. and M. Osborn 1969, M.J. Biol. Chem. 344:4406.