ported earlier (*5*), a large fraction of *Tetrahymena* phosphonolipids is further stabilized by the presence of an ether bond replacing the more easily hydrolyzed fatty ester linkage. The percentage of ciliary lipids so constructed has not yet been determined. Nevertheless, we feel that the evidence now available supports the concept that phosphonolipids may have stabilizing roles in specialized cellular membranes.

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Ribosomes from Spores of Bacillus cereus T

Abstract. *Data from analytical ultracentrifuge studies suggest that polymers of ribosomes exist in dormant spores of* Bacillus cereus *T.*

Kobayashi *et al.* (*1*) have suggested that spores of *Bacillus cereus* T do not contain a messenger ribonucleic acid (mRNA). Kornberg and co-workers have reported that patterns suggestive of polyribosomes are found in extracts from spores of *B. megaterium* (*2*). It is possible that the spores of these two species of *Bacillus* differ from one another in this significant respect, or that the results can be explained by the different methods used, or both. Here we present additional evidence (*3*) that some of the differences can be attributed to the methods used.

Lyophilized spores of *B. cereus* T were ground in a mortar with two times their mass of washed sand and then suspended in lysing buffer [20 ml per gram of spore; 10 m*M* tris(hydroxymethyl)aminomethane, *p*H 7.5; 100 m*M* NH_4Cl; 10m*M* magnesium acetate; 4 m*M* β-mercaptoethanol; and 1.0 m*M* ethylenediaminetetraacetic acid (EDTA)]. The suspensions were centrifuged twice at 40,000*g* at 0°C for 15 minutes to eliminate cell debris. Ribosomes were harvested from the supernatant by centrifugation in the 50 rotor of a Spinco model L ultracentrifuge at 42,000 rev/min for 30 minutes at −9°C. They were washed once with standard buffer (lysing buffer minus EDTA) by suspension and recentrifugation as before. The washed ribosomal pellet was suspended in standard buffer and analyzed in the Spinco model E analytical ultracentrifuge. All preparative steps were done between 0° and 4°C to minimize degradative enzymic reactions.

The peaks of ribosomes in Fig. 1A have sedimentation coefficients of 29.3, 47.6, 66.2, 94.2, 121.5, and 142.6*S*. The material in the centrifuge cell showed an absorbancy of 30 at 260 nm. An identical preparation was treated with pancreatic ribonuclease (1 μg/ml) for 5 minutes on ice just prior to loading the centrifuge cell. The sedimentation velocity pattern (Fig. 1B) illustrates the great sensitivity to ribonuclease of the bonds responsible for maintaining the multimer pattern.

Extraction with the nonionic detergent Brij 58 (1 percent weight to volume, final concentration) caused an increase in the yield of ribosomes from spores, but did not destroy the multimer profile, suggesting that membrane contamination did not contribute to the multimer patterns. These peaks were also sensitive to low concentrations of magnesium ion. No multimers were found when washed spore ribosomes were suspended in the absence of magnesium. As the concentration of magnesium increased from 2 to 5 m*M*, increasing amounts of the multimeric components were found. Above 5 m*M* magnesium ion, the pattern remained constant to at least 20 m*M* magnesium. Thus the probability that the patterns were artifactual aggregations due to divalent cation concentration is small.

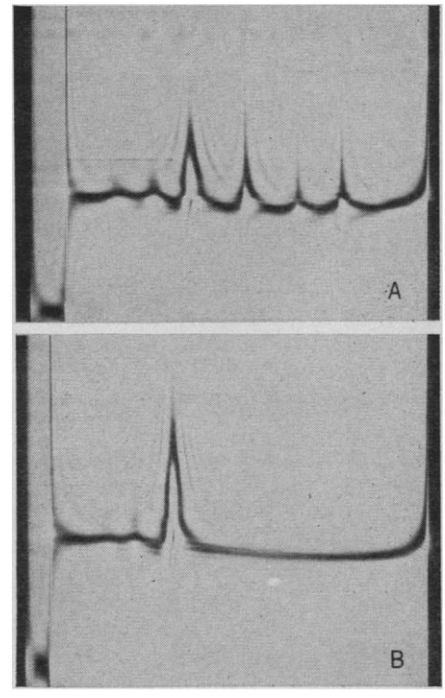

Fig. 1. Sedimentation velocity patterns obtained with ribosomes isolated from spores of *Bacillus cereus* T. Conditions of centrifugation were 40,000 rev/min; an D rotor; schlieren camera; analyzer angle, 35°; temperature of run, 20°C; and 4° sector standard kel-F centerpiece. (A) Ribosomes in standard buffer. (B) Ribosomes in standard buffer treated with pancreatic ribonuclease (1 μg/ml) for 5 minutes on ice. Sedimentation is from left to right. These pictures were taken 12 minutes after maximum speed was attained.

Ribosomal multimers are present in spores of *B. cereus* T. That they have not been seen previously is probably due to the difficulty in breaking dormant spores. The extent to which the presence of these multimers account for defects in protein synthetic characteristics of spores of this organism is still unknown.

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