Isolation of ribosomes from Neurospora and their analysis using a vertical rotor

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Abstract
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releasing the bound silver grains until only the transparent backing of the film remains. The time required for complete digestion, and hence complete clearing, was found to be proportional to the protease concentration.

In our standard method, fractions to be assayed (0.4 ml) were dispensed into 10 x 75 mm tubes, and the temperature equilibrated to 37°C. Into each of the tubes was placed a 2.5 x 35 mm strip of exposed photographic film, and the time required for complete clearing of that part of the film below the surface was determined.

The assay was calibrated with trypsin (Wellcome Laboratories), diluted when desired with 0.05 M phosphate buffer, pH 7.3. A wide range of trypsin concentrations were studied (Fig. 1). A linear plot was obtained with 0.3 to 1.25 x 10⁻³% of trypsin, but outside this range accurate determination of the end point was not possible. Even so, trypsin concentrations of 3 x 10⁻⁴% and even lower were detectable by this method.

![Figure 1](image1)

![Figure 2](image2)

Using the above method, the distribution of protease activity in a Sephacryl S-200 eluent (column size 85 x 2.5 cm, 5 ml fractions) of a crude extract of Neurospora mycelium was investigated. The results are shown in Figure 2. The proteases were eluted continuously from the column, suggesting that they were bound to molecules of much higher molecular weight (presumably their substrates), and dissociate continuously to yield free protease molecules, separated by the effects of gel filtration. The large peak of protease activity corresponds to the high molecular weight peak of aggregated proteins. (Supported by S.R.C. Grant GR/A/6465.5.)

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Isolation of ribosomes from Neurospora and their analysis using a vertical rotor.

Differential ultracentrifugation has enabled researchers to isolate and purify ribosomes. Conventional isopycnic centrifugation techniques to resolve ribosomal subunits have used swinging bucket rotors requiring long, time-consuming spins. This paper reports a new technique for ribosomal subunit separation in a sucrose density gradient by using a vertical rotor. We also report the effect of various storage conditions on the stability of Neurospora crassa ribosomes.

The Dupont Sorvall TV850 vertical rotor contains eight fixed, vertically-positioned tube apertures. During controlled, slow acceleration, tube contents are reoriented 90°, producing a very narrow sample zone and increasing the slope of the gradient. Re-orientation also significantly decreases run time due to shortening of the path length through which the sample must travel.
The experiments reported here utilized 15-25% continuous linear sucrose (Beckman, ribonuclease-free) gradients made in 50 mM Tris-HCl, pH 7.8 buffer containing 500 mM KCl, 5 mM MgCl₂ and 1 mM dithiothreitol (DTT). The gradients were refrigerated for 60 minutes at 10°C to stabilize them prior to centrifugation. The sample, ribosomes isolated from wild type Neurospora crassa and stored at -70°C (S.C. Schlitt and P.J. Russell 1974 J. Bacteriol. 120: 666-671), was thawed and immediately layered upon the gradient. Gradients were centrifuged in the TV850 rotor for 95 minutes, 47,000 rpm at 4°C. After centrifugation, gradients were displaced upwards through a flow cell to monitor nucleic acid absorbancy at 260 nm. This method of separation results in significantly greater resolution of the 60S and 37S ribosomal subunits (Fig. 1A) when compared with that achieved with the usual separation technique in which gradients are centrifuged in a Beckman SW27.1 swinging bucket rotor for 21 hours, 24,000 rpm at 4°C (Fig. 1B). Another advantage of this technique is that it overcomes the frustrating problem of partial degradation of the 37S subunit during centrifugation of gradients in the SW27.1 rotor. Quantification of this 37S degradation, using 60S:37S peak amplitude ratios (PAR) indicates that TV850 gradients result in a mean PAR of 1.5:1 and a substantially increased resolution of the 60S and 37S subunits when compared to SW27.1 gradients which result in a mean PAR of 3:1 for the two subunits.

Figure 1. -- Zone sedimentation profiles of wild type ribosomal subunits produced by various means: A. Centrifugation in TV850 vertical rotor, 95 min, 47,000 rpm; B. Centrifugation in SW27.1 swinging bucket rotor, 21 hr, 24,000 rpm; C. Maintenance of ribosome sample at 0°C for 3 hr, then centrifugation in TV850 rotor as in A.

Having established an optimal method for ribosomal subunit separation and analysis, we examined the parameters for the preparation and storage of ribosomes prior to gradient analysis. We have observed a correlation between the duration of handling ribosomes prior to layering on sucrose gradients and successful subunit separation. This led us to investigate the effects of temperature on subunit stability. After thawing, ribosomes were stored in an ice bath for 3 hours prior to layering on top of a 15-25% continuous gradient and centrifuging in the TV850 rotor as already described. A highly aberrant profile (Fig. 1C) was obtained in comparison with the control (Fig. 1A). Repeated freeze-thawing of the sample also had detrimental effects on both 37S stability and subunit separation. In summary, ribosomal subunits are quickly and efficiently separated in a vertical rotor when layered on 15-25% sucrose gradients immediately after thawing. (Supported by Grant GM-22488 from N.I.H. and NSF Grant PCM76-21478.)

Yoder, O. C.

Experience with the Applegate-Nelson-Metzenberg method of mutant enrichment in high sorbose medium.

The high-sorbose, filtration concentration method of mutant enrichment reported by Applegate et al. (Neurospora News! 25: 17, 1978) was modified and evaluated for its efficiency of mutant selection. Conidia were suspended in water, filtered through four layers of cheesecloth (20 mesh/inch), adjusted to 2 x 10⁶-2 x 10⁷/ml, and 10 ml of the suspension was placed in a 10 cm diameter glass petri dish for one min exposure to W-light (48 ergs/sec/mm²) that resulted in 70 to 90%