Rigby, D.J., T.J. Balls and A. Radford.

Semi-quantitative analysis of protease activity.

A technique has been developed in this laboratory by which Neurospora proteases con be quickly ond simply assayed semi-quantitatively. The method is particularly useful when many sampler must be assayed. The assay is bored on the digestion of the gelatin matrix of the emulsion of photographic film, digestion progressively

releasing the bound silver groins 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°. 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 (Well come 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 1.25x IO-3% of trypsin, but outside this range accurate determination of the end point was not possible. Even so, trypsin concentrations of 3x 10^{-4}% and even lower were detectable by this method.

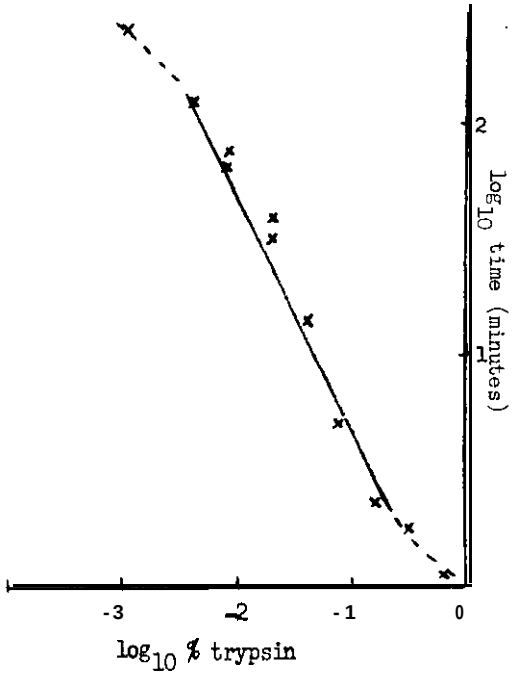

Figure 1.

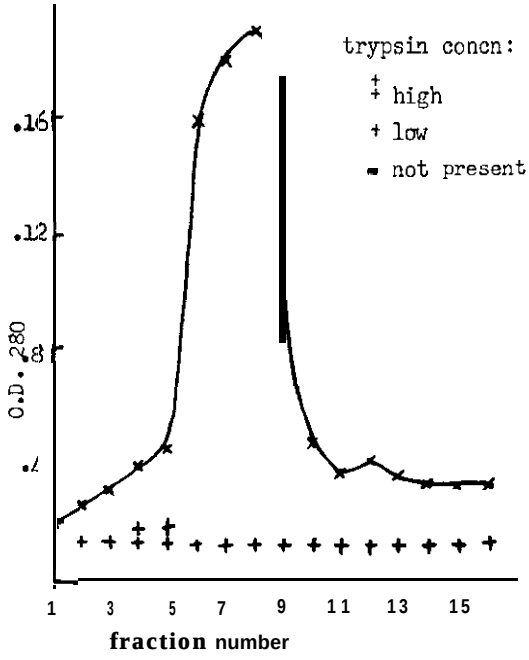

Figure 2.

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.) ▪ ▪ ▪ Department of Genetics, Leeds University, Leeds LS2 9JT, U.K.