to undertake purification on a large scale. I would appreciate hearing from other Neurosporologists if they have any suggestions in this regard.

Preliminary kinetic experiments conducted with partially purified preparations of PFK indicate that the enzyme is inhibited both by fructose-6-phosphate and ATP at higher concentrations. At least in some respects the Neurospora PFK appears to be similar to that of other microorganisms. — Department of Biology, University of Calgary, Calgary, Alberta, Canada.


Neurospora crassa strain pe Y8743m (FGSC #37) was used as a source of glutamine synthetase. Lyophilized mycelial powders were prepared as described for phosphofructokinase above. Extraction and purification were carried out at 3°C in a cold room. Twenty-five grams of the powder was extracted with 500 ml of 0.05 M phosphate buffer (5 x 10^{-4} M in EDTA and 10^{-4} M in β-mercaptoethanol), pH 7.5 for 30 min. The mixture was strained through four layers of cheese-cloth and the supernatant was centrifuged at 15,000 rpm for 15 min in a Sorvall RC-2 refrigerated centrifuge. The supernatant from this step was treated with a saturated solution of ammonium sulfate and the precipitate formed dissolved in the original buffer and further fractionated by adsorption on alumina Cy gel, as described in a recent publication (Kapoor and Bray 1968 Biochemistry 7: 3583). Elution from the gel was carried out according to our published procedure with the modification that the elution buffer was made 3 x 10^{-3} M with regard to MgCl₂. The addition of Mg²⁺ leads to a much more efficient elution and in addition to stabilization of the enzyme. The gel eluates can be stored in the form of lyophilized powder for several weeks without any loss of activity. The powder is dissolved in a small quantity of water and passed through a column of Sephadex G25 equilibrated against the original buffer containing Mg²⁺. The enzyme obtained at this stage shows an increase of 20-fold in specific activity over that of the crude extracts.

In the next step, the enzyme preparation is purified by ion exchange chromatography on a DEAE-sephadex column (2.5 x 31 cm) equilibrated with 0.02 M phosphate buffer with β-mercaptoethanol 5 x 10^{-4} M, EDTA 5 x 10^{-4} M, ribitol 0.1 M, MgCl₂ 10^{-3} M (pH 7.7). Approximately 60 mg of protein is applied on the column and washed down with buffer. Elution is then carried out by means of a linear gradient of 0-0.6 M NaCl prepared in the above-mentioned buffer. Five ml fractions are collected with an effluent flow rate of 1.0 ml per minute. The enzyme appears towards the end of the gradient, preceded by a major non-enzymatic protein peak. Without ribitol and Mg²⁺ in the eluting buffer, all enzyme activity is lost within a few hours of collection of the fractions; but ribitol stabilizes the enzyme considerably. The fractions containing enzyme activity are pooled and concentrated by ultrafiltration, using Amicon Diacrofia Ultrafilter with XM-50 membrane. This gives an enzyme preparation with an increase in specific activity of ca. 100 to 150-fold over that of the crude extract. The peak fractions show a much higher specific activity but, again, a loss occurs during concentration of the enzyme preparation. The addition of sorbitol has proved very useful in this case also. — Department of Biology, University of Calgary, Calgary, Alberta, Canada.

Eveleigh, D. E. and J. J. Child. Use of non-ionic substrates for the determination of cellulose (Cx).

Cellulose has been intensively studied in a wide range of organisms (Norkram 1967 Adv. Appl. Microbiol. 9: 91), and has recently been investigated in Neurospora from both an industrial (Kuroda 1968 Chem. Ab. 68: 1895Sd) and a theoretical standpoint (Meyers and Eberhard 1966 Biochem. Biophys. Res. Commun. 24: 782). Although there have been attempts to rationalize the cellulose assay (β-glucosidase activity) on an absolute basis (bonds broken per second = Almin and Eriksson 1968 Arch. Biochem. Biophys. 124: 229), there is a lack of standardization between published methods. For example, carboxymethyl cellulose (CMC) has been used in several different states each with various degrees of substitution (DS) and of polymerization (DP), which control the rate of the reaction. Its ionic character limits its use in viscometric assays, as the viscosity is dependent on pH, ionic strength and polyvalent cation content of the assay medium. There are limitations magnified in the more enzymically reactive, lower substituted CMC's. Glycolic cellulose (hydroxy ethyl cellulose = H EC) has been proposed to replace CMC in order to circumvent these difficulties (Iwasaki et al. 1964 J. Biochem. (Tokyo) 55: 30) but has been rarely used, presumably because there authors noted the laborious procedure of making this non-ionic substituted cellulose. A rigorously controlled range of H EC's is available commercially and this allows the facile development of more standardized reductometric and viscometric cellulose assays. For this purpose we have used the readily soluble Natrosol 250 H EC (4,500-6,500 centipoises at 2% mean substitution 2.5, DS ca. 1.0, DP 565 = Hercules Powder Co., Wilmington, Delaware). Equivalent H EC's are available from Farberweike Hoechst A. G., Frankfurt, Germany (Tylose H 4000) and Union Carbide Corp., New York (Cellosolve WP 4400H). Viscometric assays were carried out using a Cannon-Fenske Viscometer (No. 200: efflux time of solvent 10.2 sec.) at 25°C with a reaction mixture of 7 ml 0.44% HEC (250 M Natrosol), 1 ml 0.5 M sodium acetate buffer pH 4.8 and 2 ml enzyme. The substrate was dissolved in water by shaking overnight at room temperature. Blending to aid the rapid solution of the substrate for at least five seconds gave a much reduced viscosity level. Reductometric analyses were performed using equivalent reaction mixture, reducing end groups being estimated by the ferricyanide method modification proposed by Pork and Johnson (1949 J. Biol. Chem. 171: 149). The calorimetric Somogyi-Nelson method could not be used as it caused precipitation of the substrate and concomitant absorption of the colored complex. Titrimetric analyses of the oligosaccharides by the Somogyi method or by direct reduction with alkaline iodine proved practical but tedious. Enzyme units are defined: one reductometric unit is that amount of enzyme which producer 1% degradation in 1 minute, under the above conditions, while a viscometric unit is that amount of enzyme which causes a change of Δf (specific fluidity) of 0.10 in 10 minutes when incubated under the above conditions. Apparent zero order kinetics were maintained up to a change of Δf of 0.10. It is important to note that substituted celluloses are degraded at a changing rate...