

Tsao, M. U. and M. W. Smith. Multiple forms of glucose-6-phosphate dehydrogenase of *Neurospora crassa*.

property of the enzyme in this organism rather than an anomalous manifestation of the crude preparation, purification procedures have been applied. These include protamine treatment, ammonium sulfate precipitation, DEAE column chromatography, and dialysis. Products from each step, as well as the crude extract, were simultaneously subjected to vertical electrophoresis in starch gel and separated bands of enzymic activity were detected in the gel.

Fernbach flasks containing 1 liter of Difco *Neurospora* medium are inoculated with a small piece of mycelium of *N. crassa* 10336 (ATCC). The flasks are incubated at 30° without shaking for 1 day and are then shaken 29 hours. The mycelium is harvested, washed, and frozen. This is followed by lyophilization and grinding in a Wiley mill. The powder that passes 60 mesh is extracted with acetone (-20°) and the defatted powder is homogenized in dilute phosphate buffer of pH 6.7. The centrifuged homogenate is used as crude extract. Protamine sulfate is added to the crude extract and the precipitate is removed by centrifugation. The supernatant is called protamine-treated extract.

The precipitate that comes out between 40 and 55% saturation with ammonium sulfate of the protamine-treated extract contains most of the enzymic activity. The protamine-treated extract is also concentrated by dialysis against sucrose crystals and the excess sucrose is removed by dialysis against phosphate buffer. The DEAE column is equilibrated with phosphate buffer and loaded with concentrated enzyme preparation. The column is eluted with phosphate buffer with continuously rising NaCl concentration. The fraction containing the enzyme is concentrated by dialysis against sucrose.

The starch gel is prepared with 0.03 M Tris buffer of pH 8.5. Vertical electrophoresis is carried out in a refrigerator at 0°, at 5 v/cm for 20 hours. The sliced gel is placed in a bath of 0.05 M glycyl-glycine buffer of pH 7.6 containing:  $4 \times 10^{-4}$  M glucose-6-phosphate,  $3.6 \times 10^{-5}$  M TPN,  $2.5 \times 10^{-3}$  M  $MgCl_2$ ,  $10^{-3}$  M KCN, 20 mg% phenazine methosulfate, and 50 mg% nitro-blue tetrazolium.  $N_2$  atmosphere is used for the incubation to bring out the bands of enzymic activity.

The persistence of three electrophoretically distinct bands of enzymic activity in the starch gel after purification procedures shows that glucose-6-phosphate dehydrogenase of *Neurospora crassa* exists in multiple forms. Furthermore, these forms of enzyme are so similar in their properties that they have so far not been separable by other than electrophoresis in the starch gel. (Work supported by National Science Foundation.)--University of Michigan, Ann Arbor, Michigan.

Preliminary observation in this laboratory of crude extracts of *Neurospora crassa* indicated multiple forms of glucose-6-phosphate dehydrogenase (Science 136, 42 (1962)). To confirm this observation as a true