Preparation of an active glucose 6-phosphate dehydrogenase from *Neurospora crassa*

During investigations of the biosynthesis of valine and isoleucine in *Neurospora crassa*[1], commercial samples of TPNH prepared by reduction of TPN by $Na_2S_2O_4$ were found to inhibit severely α-keto-β-hydroxy acid reductase. Crude extracts of *N. crassa* contained an active glucose 6-phosphate dehydrogenase and offered a convenient source of this enzyme for the enzymic preparation of TPNH. Purification of the enzyme has yielded fractions of high specific activity with reasonable recoveries.

The assay mixture consisted of enzyme fraction, suitably diluted, 0.1 ml; glucose 6-phosphate, 1.0 μmole; TPN, 0.1 μmole; Tris buffer, pH 7.5, 100 μmoles; all in a total volume of 1.0 ml. The increase in absorbancy was measured at 340 mμ and 25 to 27° in a recording spectrophotometer. A unit of enzyme activity[2] is that amount of enzyme that produces a change of 1.000 absorbancy unit/min under the assay conditions. 6-Phosphogluconate dehydrogenase was measured under the same conditions, but with 6-phosphogluconate replacing glucose 6-phosphate as substrate.

Mycelium from a 60-h culture of *N. crassa* (16117 A) grown with shaking at 37° in a synthetic medium[3] was filtered, ground in a mortar under liquid nitrogen, then homogenized in a Ten Broeck apparatus with about twice its volume of 0.1 *M* phosphate buffer, pH 7.0. This extract (Fraction A, 400 ml, pH 6.8) was treated with a 1.5 % solution of protamine sulfate in 0.1 *M* phosphate buffer, pH 7.0, at the rate of 15 mg protamine sulfate/100 mg protein[4]. After stirring for 15 min, the precipitate was removed by centrifugation. The supernatant solution (Fraction B; 440 ml) was adjusted to pH 7.0, 77.4 g $(NH_4)_2SO_4$ were added, and the pH was readjusted to 7.0 by the addition of NaOH. After 30 min, the precipitate was removed by centrifuging. To the supernatant solution (482 ml), 29.9 g $(NH_4)_2SO_4$ were added, the pH was adjusted to 7.0 with NaOH, and after 30 min the precipitate was again removed by centrifuging. The supernatant solution (488 ml) was finally treated with 30.7 g $(NH_4)_2SO_4$ and the pH again adjusted to 7.0 and the mixture centrifuged after 30 min. The precipitate contains the dehydrogenase and was dissolved in 42.0 ml 0.1 *M* Tris buffer, pH 7.5 (Fraction C). This fraction contained about 62 % of the glucose 6-phosphate dehydrogenase activity originally present in the crude extract and did not dehydrogenate 6-phosphogluconate.

Fraction C (40 ml) was dialyzed for 12–15 h against 6 l 0.005 *M* phosphate buffer, pH 7.5, containing 10^{-4} *M* Mg^{++}. The precipitate formed during dialysis was discarded. The volume of the supernatant solution was adjusted to 80.0 ml with cold distilled H_2O (Fraction C-1). The pH was adjusted to 6.5 by careful addition of 0.1 *N* acetic acid and the precipitated protein removed by centrifuging. The supernatant solution (Fraction C-2) was subjected to fractional adsorption with calcium phosphate gel (OCHOA *et al.*[5]). 10 ml of centrifuged gel (15.4 mg solids/ml) and an absorption time of 15 min, with stirring, were used for each of six steps. Each gel precipitate thus obtained was eluted by stirring for 30 min with 40.0 ml 0.2 *M* phosphate buffer, pH 8.0, and the supernatant solution transferred to the next gel step, in sequence[5]. All operations were carried out at 0–5°. Most of the glucose 6-phosphate dehydrogenase was present

Abbreviations: TPN, TPNH, oxidized and reduced triphosphopyridine nucleotide; Tris, tris(hydroxymethyl)aminomethane.

TABLE I

SUMMARY OF PURIFICATION PROCEDURE FOR THE GLUCOSE 6-PHOSPHATE AND 6-PHOSPHOGLUCONATE DEHYDROGENASES FROM *N. crassa*

*Fraction**	*Volume ml*	*Protein mg/ml*	*Glucose 6-phosphate dehydrogenase units/mg protein*	*6-phosphogluconate dehydrogenase units/mg/protein*
A	400	12.1	1.95	0.63
B	440	9.0	2.54	0.87
C	42	18.7	7.55	0
C-1	80	6.2	13.3	
C-2	82	4.8	14.2	
C-3	40.5	1.29	35.6	
C-4	40.5	1.10	36.3	
D	42.5	17.3	4.27	1.15
E	46.0	21.7	0	1.15

* See text.

in gel eluates 4 and 5 (Fractions C-3 and C-4, Table I) and the combined recovery in these fractions was 37 %.

Activities of the best fractions (Table I) are higher than those reported for many preparations of glucose 6-phosphate dehydrogenase[2,6,7], and the yield is more satisfactory. No specific requirement for Mg^{++} was found, and under the assay conditions DPN does not substitute for TPN.

Although free of 6-phosphogluconic acid dehydrogenase, the preparation contains hexokinase activity equivalent to 14.5 % of the glucose 6-phosphate dehydrogenase activity. The presence or absence of other closely associated enzymes has not been investigated.

The supernatant solution from Fraction C contained 6-phosphogluconate dehydrogenase. This enzyme could be separated from glucose 6-phosphate dehydrogenase by successive additions of 32.7 g and 114.6 g, respectively, of $(NH_4)_2SO_4$ to precipitate fractions D and E of Table I.

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