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Short Communication

Studies on the isolation of glucose-6-phosphate dehydrogenase from *Aspergillus nidulans*

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Abstract

The effect of different cultural conditions on the level of activity of G6PDH in *Aspergillus nidulans* was studied. The enzyme was isolated from this organism, partially purified by ammonium sulphate fractionation and DEAE cellulose chromatography and its molecular weight determined. Kinetics of this enzyme was studied.

Key words: G6PDH, *A. nidulans*, kinetic properties, isolation.

1. Introduction

Hankinson and Cove[1] have shown that nitrate in the medium increased the HMP pathway activity in *Aspergillus nidulans* as perhaps nitrate reductase and hydroxylamine reductase use only $NADPH_2$ as coenzyme in this organism. Since glucose-6-phosphate dehydrogenase is the first enzyme in this pathway, an attempt has been made to characterise it from *Aspergillus nidulans* and compare its properties with the enzyme isolated from other microorganisms.

2. Materials and methods

The wild strain of *Aspergillus nidulans* bearing green conidia was obtained from the Glasgow stock. Glucose-6-phosphate, NADP and PMS (phenazine metho sulphate) were obtained from Sigma, USA. All other chemicals used were of analytical grade.

Minimal medium was prepared according to the method of Pontecorvo *et al*[2]. For isolation of the enzyme one litre of the medium was dispersed into several Haffkine's flasks, inoculated with a spore suspension of *A.nidulans* and grown for 3 days at 37°C. Enzyme assay was carried out according to the method described by Ells and Kirkman[3]. One unit of enzyme activity is defined as that amount of enzyme which catalyses the reduction of one μ mole of the substrate per minute.

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2.1. *Isolation of the enzyme*

The mycelia was washed (free of adhering medium) with distilled water and buffer (0·01M Tris-HCl, pH 7·4), extracted with 20 volumes of buffer per g of mycelia in a mortar and pestle and stirred slowly for 2 – 3 hours; centrifuged for 10 minutes at 2500 × g and collected the supernatant. This step and all other subsequent steps were carried out at 4°C. The buffer used in further steps contained 2×10^{-5} M NADP and 1mM β–mercapto ethanol. The former protected the enzyme from heat inactivation and the latter increased the yield of the enzyme during purification.

Ammonium sulphate (209 g/l) was added to the crude extract, and the precipitate, collected by centrifugation at 12,000 × g for 10 minutes, was discarded. To the supernatant, 164 g of ammonium sulphate per litre of the original crude extract was added and the precipitate collected by centrifugation as earlier. It was dissolved in buffer (0·1M Tris-HCl, pH 7·4) and dialysed against the same.

The solution after dialysis was adsorbed onto a DEAE-cellulose column (1 ml of exchanger for 5 – 7 ml of protein) and eluted at the flow rate of 5 ml/cm^2 per hour using a linear gradient of NaCl, 0·05 M to 0·2 M in 0·1 M Tris-HCl buffer, pH 7·4. Fractions containing enzyme activity were pooled and the enzyme precipitated by 472 g of ammonium sulphate per litre; the precipitate was collected by centrifugation, dissolved in buffer and dialysed against the same.

A second DEAE-cellulose column chromatography was performed wherein the protein load was reduced to 1 mg per ml of exchanger and the flow rate was reduced to 4·5 ml per cm^2 per hour. The enzyme was eluted using NaCl of ionic strength 0·05 to 0·2 M. The pH of the buffer used was 8·3. The enzyme was precipitated with ammonium sulphate as in the earlier step, collected by centrifugation, dissolved in buffer and dialysed against the same (0·1 M Tris-HCl, pH 8·3).

Molecular weight of the enzyme was determined by the method of Andrews[4]. Polyacrylamide gel electrophoresis was carried out by a modification of the procedure of Davis[5]. The gel was stained for activity with phenazine metho sulphate nitroblue tetrazolium staining of Tsao[6] and for protein with Amido Schwarz.

3. **Results**

It was observed that glucose, maltose or sucrose can be used as carbon source and sodium nitrate or ammonium salts as nitrogen source for G6PDH production by *A.nidulans*. The enzyme production was maximum (52 units) when grown at 37°C. for 3 days (data not shown).

Table I gives the data on purification of the enzyme. The enzyme protein could be eluted as a single peak in DEAE-cellulose column.

The enzyme has a broad pH optimum of 7·3 to 8·1. The characteristics studies carried out show that the Km for G-6-P was 2×10^{-5} M and $7{\cdot}7 \times 10^{-5}$ M for NADP. NAD cannot replace NADP as coenzyme. The enzyme exhibited high stereospecificity with

Table I
Purification of G6PDH from *Aspergillus nidulans*

Fraction	Total protein (mg)	Total activity (units)	Specific activity	Yeild (%)
Crude extract	7339	79034	10	100
Ammonium sulphate precipitation 60%	1286	45511	35	59
DEAE-Cellulose chromatography pH 7·4	101	40511	401	51
Ammonium sulphate precipitation 70%	31	24545	791	31
DEAE-Cellulose chromatography pH 8.3	13	20000	1538	25
Ammonium sulphate precipitation 70%	3	12102	4071	15

respect to glucose-6-phosphate, only very low activities being demonstrable with galactose-6-phosphate or fructose-6-phosphate. The molecular weight of the enzyme was found to be around 190,000. The enzyme appears to be an −SH enzyme since it was inhibited by PCMB. The enzyme activity was inhibited by mercury, lead, phosphate and ATP and was stimulated by Ca^{++}, Mn^{++}, Mg^{++} and Cys.HCl (data not shown). The enzyme was inactivated by freezing and was active for two weeks at 4°C.

4. Discussion

The enzyme resembles the one detected in *C. utilis* as far as the Km is concerned[7]. It has stereospecificity for glucose-6-phosphate, whereas the enzyme isolated from yeast[8] and *N. crassa*[9] can use galactose-6-phosphate and 2-deoxy glucose-6-phosphate also as substrates. In its cofactor requirements it resembles the enzyme isolated from *N. crassa*[9] and *P. duponti*[10] and differs from the enzyme isolated from *L. mesenteroides*[11], *P. aeruginosa*[12] and *B. licheniformis*[13] which can use both NAD and NADP as cofactors. Isoenzymes for G6PDH have been reported in *N. crassa*[9] and *C. cresenticus*[14]. In *A. nidulans* only one band was obtained.

5. Conclusions

Thus the enzyme isolated from *A. nidulans* can act only on glucose-6-phosphate, is a −SH enzyme and the increase in activity parallels growth suggesting that this enzyme may play a key role in cellular synthesis in this organism.

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