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Isolation of myo-inositol-1-phosphate synthetase from wild type and an inositol-less variant Neurospora crassa

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Isolation of myo-inositol-1-phosphate synthetase from wild type and an inositol-less variant Neurospora crassa

Abstract

Isolation of myo-inositol-1-phosphate synthetase from wild type and an inositol-less variant

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Isolation of myo-inositol-1-phosphate synthetase from wild type and its inactive variant from inositol-less Neurospora crassa.

An inositol dependent mutant of Neurospora crassa was used as a recipient to study transformation in eukaryotes. This mutant lacks myo-inositol-1-phosphate synthetase (MIPS, E.C. 5.5.1.4) activity. The defect can be "transformed" by the addition of high molecular weight DNA isolated from the wild type (Mishra et al. 1973 pp. 259-268. In Niu and Segal (eds.), The role of RNA in reproduction and development. North-Holland Publ., Co., Amsterdam). To elucidate the biochemical nature of inositol dependency and to help understand the process of "transformation", we studied the properties of MIPS from the wild type strain (RL-3-8 A) and compared it to the homologous defective protein, if any, of the inositol-less mutant (R-2506-8-12).

MIPS was isolated from the wild type strain according to Pina and Tatum (1967 Biochim. Biophys. Acta 136:265; Chen and Charalampous 1965 J. Biol. Chem. 240: 3507; 1966 Arch. Biochem. Biophys. 1117: 154) with modifications. Our procedure utilizes protamine sulfate and ammonium

Table 1

Change of protein content and enzyme activity during the isolation of myo-inositol-1-phosphate synthetase

Isolation step	Total protein* /mg/	Total activity** /units/
Crude extract	4 200	
100 000 g supernatant	3 040	186 000
Protamine sulfate supernatant	2 630	200 000
Amonium sulfate franction /0.5-0.67 saturation/	370	100 000
Sephadex G 200 chromatography	110	85 000
DEAE-Sephadex A 25 chromatography	13	60 000
Final product	12	57 000

*Protein content was determined by biuret method as well as by measuring the absorbancy at 380 nm.

**Enzyme activity was determined with glucose-6-phosphate as substrate at pH7.7 according to Pino and Tatum (1967), but by measuring the inorganic phosphate released by periodate oxidation of inositol-1-phosphate (Barnett et al. 1970 Biochem. J. 119: 183).
One unit of activity is expressed as 1 nmol P_i released per hour per ml of assay mixture.

sulfate fractionation followed by gel filtration and ion exchange chromatography.

Mycelia were extracted with 0.1 M Tris-HCl, pH7.7, containing protease inhibitors (1975 Siepen et al. Eur. J. Biochem. 56: 271). Starting with about 20 g wet weight, 186 000 enzyme units were found in the original 100 000 g supernatant, and 57 000 units (i.e. about 30 percent) was found in the final product (Table 1). The enzyme purity had increased about 100-fold compared to the 100 000 g supernatant.

Gel filtration on Sephadex G-200 column subsequent to salt fractionation appeared to be suitable to remove phosphatases. The MIPS activity was eluted directly after the exclusion volume, whereas phosphatases appeared in the later fractions. During chromatography on DEAE-Sephadex A-25 we used a linear gradient at pH7.7 and MIPS was eluted between 0.15-0.25 Tris-HCl.

The molecular weight of the MIPS was 225 000, as determined by gel filtration. The subunit composition was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Two protein bands were obtained, their molecular weights were 64 000 and 50 000. It seems probable that the enzyme is a tetramer containing 2 copies each of two different subunits. Pino et al. (1969 Annal. N.Y. Acad. Sci. 165: 541) reported that MIPS from N. crassa has a molecular weight of 150 000 but in our case the sedimentation constant (8.0 S) and subunit analysis suggested a higher molecular weight.

Using exactly the same procedure we isolated a protein fraction which had no enzyme activity from the inositol dependent mutant. This protein fraction was compared by polyacrylamide gel electrophoresis with wild type MIPS.

The enzyme preparation contained, in addition to the enzymatically-active main band, several accompanying proteins in small amounts. The protein fraction isolated from the mutant also yielded several protein bands, but at the precise location of the active enzyme, a well defined band appeared.

In preliminary experiments this protein has shown a positive reaction with immune sera produced in mice against the purified MIPS. This suggests that the protein isolated from the inositol-less mutant is a defective variant of the MIPS found in the wild type. (This work was supported by grants of the Hungarian Ministry of Health and the Hungarian Academy of Sciences). - - - Departments of Biochemistry and Biology, Central Research Laboratory, University Medical School of Debrecen, H-4012 Debrecen, POB 6, Hungary.