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Regulatory effect of inasitol on the synthesis of myo-inositol-l-phosphate synthase in Neurospora crassa strains

The inositol-synthesizing enzyme myo-inositol-1-phosphate synthase (MIPS, EC 5.5.1.4:) converts glucose-6-phosphate into inositol-l-phosphate. It has been isolated in a highly purified form and its molecular properties have also been determined (Zsindely et al., 1977 Acta 11. Acad. Sci. hung. 28: 281; Aradi et al ., 1980 Neurospora Newsl. 27: 27). The production of a cross-reacting defective protein has been detected by immunological methods in inositol-requiring mutants which are characterised by lack enzyme activity (Zsindely et al., 1979 Acta biol. Acad. Sci. hung. 30: 141).

In the present experiments the biosynthesis of the enzyme and that of the defective protein were studied in various strains of Neurospora crassa. Enzyme activity was measured in crude extracts of cultures by analyzing quantitatively the production of inositol-l-phosphate as described by Barnet et al. (1970 Biochem. J. 119: 183). The amount of proteins reacting with monovalent imnune-sera produced against highly-purified enzyme was determined by rocket immunoelectrophoresis according to Laurel1 (1966 Anal. Biochem. 15: 45) in a

1% agarose gel containing 1% immune-serum. The 5 µl samples contained 50-100 µg of total protein. The height of the precipitation peaks (0.5 - 2.5 µg antigen) is proportional to the antigen content.

Using various strains of Neurospora crassa in different phases of the vegetative growth, we found that enzyme production is dependent upon the age of the culture. Hence, for the following experiments, cultures in exponential growth were used.

Eighteen-hours, wild type cultures showed that the production of MIPS decreased in the presence of inositol in the medium. In crude extracts, enzyme activity as well as the antigen content, decreased in parallel with the increasing concentrations of inositol (Table I). In control experiments, enzyme activity of crude extracts was not influenced by the addition of inositol at the same concentrations during growth.

An inositol-requiring mutant (89601) was grown in medium supplemented with 100 µg/ml inositol. Under such conditions a significant amount of a cross-reacting material, i.e. a defective protein, could be detected in the crude extracts. The amount of the antigen did nor change by decreasing the concentration of inositol to 12.5 µg/ml in 24-hr and 36-hr cultures (Table I).

To see if the native conformation of MIPS is needed for inositol inhibition, we studied a thermosensitive inl mutant (FGSC #2257), which grows only with inositol at 37°C. In 2O-hr cultures grown at 22°C, an increase in the concentration of inositol brought about a marked decrease in enzyme activity (as compared to the control), whereas the amount of the cross-reacting antigen was hardly reduced (Table II).

Although the present experiments do not elucidate the mechanism of regulation, it can be concluded that inositol or its derivative inhibits the production of the enzymatically-active MIPS in the wild type, i.e. it is effective in these strains on the level of gene regulation. However, the results obtained with the thermosensitive mutant suggest that regulation at the enzyme level should also be considered, i.e. the assembly of the synthesized precursors producing the active enzyme can somehow also be influenced by inositol.

TABLE I

Effect of inositol on the production cf myo-inositol-l-phosphate synthase and the defective protein in different strains

1nositol (µg/ml medium)	WILD-TYPE STRAIN[a]			INL MUTANT[b]	
	Activity (U/mg protein)	Antigen (µg/mg protein)	Activity / Antigen	Activity[c] (U/mg protein)	Antigen (µg/mg protein)
0	76.1	30.5	2.50		
12.5	NDd	ND			38.6
25.0	38.8	15.0	2.58		38.0
50.0	26.0	10.6	2.45		40.0
75.0		7.5	0	±	40.0
100.0	±	6.2	0		38.2

[a] Strain RL-3-B was cultivated for 18 hours.
[b] Strain 89601 was cultivated for 36 hours.
Enzyme activity, antigen and protein content were determined in the crude extracts obtained after centrifugatian (100 000 g) and dialysis.
[c] Enzyme activities below 10 U/mg protein are denoted by ±.
[d] Not done.

TABLE II

Inhibition of myo-inositol-l-phosphate synthase formation by inositol in a thermosensitive mutant[a]

Inositol (µg/ml medium)	Activity (U/mg protein)	Antigen content (µg/mg protein)	Enzyme activity / Antigen content
0	48.0	57.0	0.84
25	16.2	47.5	0.34
50	12.1	44.5	0.27
100		45.0	

[a] Strain FGSC #2257 was cultivated at 22°C for 20 hr and the results were calculated in Table I.

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