Fungal Genetics Reports

Volume 35 Article 7

Effect of inositol "analogues" on the production of myo-inositol-1-phosphate synthase in Neurospora crassa slime strain.

A. Kiss

M. Schablik

A. Zsindely

See next page for additional authors

Recommended Citation

Kiss, A., M. Schablik, A. Zsindely, and G. Szabo (1988) "Effect of inositol "analogues" on the production of myo-inositol-1-phosphate synthase in Neurospora crassa slime strain.," *Fungal Genetics Reports*: Vol. 35, Article 7. https://doi.org/10.4148/1941-4765.1527

Effect of inositol "analogues" on the production of myo-inositol-1-phosphate synthase in Neurospora crassa slime strain.

Abstract

Effect of inositol "analogues" on the production of myo-inositol-1-phosphate synthase in Neurospora crassa slime strain.

Authors

A. Kiss, M. Schablik, A. Zsindely, and G. Szabo

Kiss, A.^1, M. Schablik^2, A. Zsindely^1 and G. Szabo^2

Effect of inositol "analogues" on the production of myo-inositol-l-phosphate synthase in Neurospora crassa slime strain.

The myo-inositol-1-phosphate synthase (MIPS, E.C. 5.5.1.4) synthesis is regulated by at least two regulatory genes, inl^ts and opi-1. Zsindely et al. (1983 Biochem. Biophys. Acta 741:273-278) assumed that inl^ts is a positive regulatory gene, responsible for the production of a thermosensitive protein. Mutations in the inl^ts gene dramatically decrease or completely turn off MIPS production (unpublished results), and a mutation of the regulatory gene opi-l derepresses MIPS production (Kiss et al. 1986 Fungal Genetics Newsletter 33:29-30).

It has been presumed that non--metabolized inositol analogues might influence the MIPS synthesis. The effect of gamma- and delta-hexachlorocyclohexane (HCH) was examined upon MIPS production. The Neurospora crassa slime (FGSC 1118 inl^+) variant growing in the form of spheroplasts was applied in the experiments.

The slime strain was grown in Nelson B medium in submerged culture (100 rpm at 27°C for 22 hours. HCH stereoisomers were dissolved in 83% ethanol (1.0 mg.ml) and were added to the medium between 0-8 ug/ml. Ethanol in <0.6% concentration does not influence the growth of the strain and MIPS synthesis (unpublished result).

The growth of the cultures was followed by determination of the number of cells. The 100,000 g supernatants of crude extracts (Zsindely et al. 1977 Acta Biol. Acad. Sci. Hung. 28:281-290) were used to determine enzyme activity by the modified method of Barnett et al. (1970 Biochem. J. 119:183-186). Immune sera raised against highly purified MIPS were used to detect enzyme content. Rocket immunoelectrophoresis was carried out according to Laurell (1966 Anal. Biochem. 15:45-49). Protein concentration was determined by the micro-biuret technique (Itzhaki and Bill 1964 Anal. Biochem. 9:401-410).

Table I shows there is a 40% inhibition of cell number accumulation at 4 ug/ml gamma-HCH while at 8 ug/ml it is 75%. delta-HCH at 4 ug/ml inhibits by 85%. At higher delta-HCH concentration there is no growth at all. MIPS synthesis is enhanced by both stereoisomers but to different extent. At 8ug/ml gamma-HCH, the quantity of the enzyme increases by 20%. 4 ug/ml delta-HCH raises the quantity of the enzyme by 120%. The specific activities are independent of the effect of the analogues. They do not inhibit the enzyme already formed. Accordingly, both gamma-HCH (muco) and delta-HCH (myo-inositol) isomers influence MIPS production, probably by affecting gene expression. The effect of the myo-inositol structural analogue delta-HCH upon MIPS production is especially enhanced.

Table I. Effect of gamma- and delta-hexachlorocyclohexane on the growth and MIPS production of Neurospora crassa slime strain

analogue conc.	gamma-HCH				delta-HCH			
ug/ml medium	A	B	C	D	A	B	C	D
0	49	67	0.73	7.6	44	59	0.75	5.5
1		n.d.			45	61	0.74	3.7
2	45	61	0.74	7.2	48	65	0.74	1.0
4	46	63	0.73	4.5	96	130	0.74	0.1
6	65	87	0.75	2.8			n.d.	
8	67	90	0.74	1.9			n.d.	

Cultivation at 27° C, 22 h. MIPS determination in the 100,000 g supernatant of crude extracts.
A: MIPS activity U/mg protein B: antigen content (pg/mg protein)
C: specific activity U/pg antigen D: cell number x 10^6
n.d. not determined

- - - Departments of Biochemistry^1 and Biology^2, University Medical School of Debrecen, H-4012 Debrecen POB 6 Hungary