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Effect of inositol "analogues" on  
the production of myo-inositol-1-  
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 Mutations in the inl^ts gene dramatically decrease or completely turn off MIPS production (unpublished results), and a mutation of the regulatory gene opi-1 derepresses MIPS production (Kiss et al. 1986 Fungal Genetics Newsletter 33:29-30).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0.6% concentration does not influence the growth of the strain and MIPS synthesis (unpublished result).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<br>conc .<br><br>ug/ml<br>medium | gamma-HCH |    |      |     | delta-HCH |     |      |     |
|-------------------------------------------|-----------|----|------|-----|-----------|-----|------|-----|
|                                           | 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<sup>6</sup>  
n.d. not determined