Fungal Genetics Reports

Volume 25 Article 20

Detection and identification of myo-inositol-1-phosphate synthase and its assumed defective variant in different Neurospora crassa strains by immunological methods

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Recommended Citation
Zsindely, A., M. Szabolcs, M. Kavai, M. Schablik, J. Aradi, and G. Szabó (1978) "Detection and identification of myo-inositol-1-phosphate synthase and its assumed defective variant in different Neurospora crassa strains by immunological methods," *Fungal Genetics Reports*: Vol. 25, Article 20. https://doi.org/10.4148/1941-4765.1751

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Abstract

Detection and identification of myo-inositol-1-phosphate synthase and its assumed defective variant in different *Neurospora crassa* strains by immunological methods

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This research note is available in Fungal Genetics Reports: http://newprairiepress.org/fgr/vol25/iss1/20

Zsindely, A., M. Szabolcs, M. Kavai, M. Schablik, J. Aradi and G. Szabó.

Detection and identification of myo-inositol-1-phosphate synthase and its assumed defective variant in different Neurospora crassa strains by immunological methods.

Inositol requiring mutant inl (89601); rg (R2357) a(strain R2506-8-12) of N. crassa lacks myo-inositol-1-phosphate synthase activity (MIPS, E.C.5.5.1.4). The defect can be "cured" by addition of high molecular weight DNA isolated from wild type strain (RL3-8 A) (Mirhro et al. (1973) pp. 259-268. In Niu and Segal (eds.), The role of RNA in reproduction and development. North-Holland Publ. Co., Amsterdam; Szabó and Schablik (1975) Neurospora Newsl. 22: 11). A procedure has been published for the isolation of MIPS from wild type strain and some of its properties have described (Zsindely et al. (1977) Neurospora Newsl. 24: 8-9; (1977) Acta Biol. Acad. Sci. Hung. 28: 281-290). In this report the procedure is applied to the partial purification of immunologically cross reacting protein from on inl strain, a transformed (inl → in, $^{+}$) strain, and a spontaneous revertant.

The protein fraction isolated from the inl strain by the same procedure as that used to isolate MIPS from wild type hod no enzyme activity, but gel electrophoresis yielded several protein bands that ore located at approximately the same position as that active MIPS from wild type. The homologous defective protein, if any, was presumed to occur among there components.

The identification of assumed "defective form" of MIPS was attempted with immune sera produced against wild type enzyme. Mice were immunized interperitoneally with MIPS according to Tung et al. (1976, J. Immunol. 116: 676-681) with complete Freund adjuvant After three weeks the mice were given a second injection and they were treated with Ehrlich ascites tumor cells. Two weeks later the peritoneal exudates were removed. After centrifugation the clear exudates were used as "antisera" for antigen analysis. In immunodiffusion experiments performed according to Ouchterlony in agarose gels (Figure 1), MIPS resulted in a single precipitation bond and its intensity was proportional to enzyme concentration (10, 5 and 2 µg protein). A similar but much weaker precipitation band was obtained with the protein fraction isolated from inl.

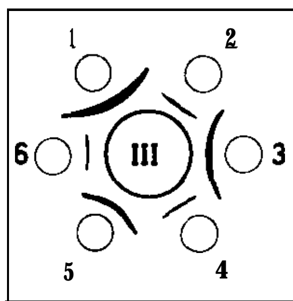

Figure 1. Immunodiffusion of MIPS and the protein fraction isolated from inl (89601). Experiment was carried out in 0.6% agarose gel, 0.1 ml antiserum III, staining with Amido-black 10 B.
Wells 1, 3, 5 contained MIPS from the wild type 10, 5 and 2 µg protein respectively.
Wells 2, 4, 6 contained the protein fraction from inl 10, 5 and 2 µg protein respectively.

To define which electrophoretic species is responsible for the immune response a procedure was developed to accomplish the immune reaction with antiserum directly in the gel tube immediately after electrophoresis. A similar method is described by Makonkawkeyoon and Haque (1970, Anal. Biochem. 36: 422-427). In the "immunodisc" electrophoretic technique acrylamide was polymerized as hollow cylinders by placing thin plexiglass rods in the middle of tubes which were immediately removed after the electrophoresis. Then one of the parallel sampler was directly stained while the other one was used for antigen analysis, i.e., the lumen was filled with antiserum diluted by saline. After immunoprecipitation other proteins that were not precipitated were removed by efficient washing and the gal also stained. The comparison of purified MIPS and the fraction isolated from the inl mutant (Figure 2) show that in contrast to the nearly homogeneous MIPS from the wild type, the protein fraction from the mutant consisted of several components, but only one of them which had a somewhat lower mobility than that of the wild type enzyme resulted in a definite immune response. In addition, a weak reaction was given by another component of a much lower mobility.

Investigations were also performed with a transformant strain, obtained by treatment with DNA of wild type (allo-DNA), and with a spontaneous revertant. A well pronounced MIPS activity was measured in the extracts of both strains and positive immune responses were detected by "immunodisc" electrophoresis. The enzymes were isolated from both strains and some properties of them were compared to MIPS from the wild type. Their specific activities were lower (transformant = 2860 U/mg protein, spontaneous revertant = 986 U/mg protein, while their Michaelis constants for the substrate were to some extent higher (2.4 mM and 2.6 mM) than those of MIPS from the wild

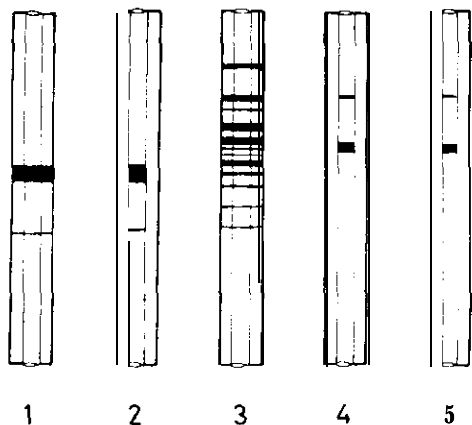

Figure 2. Immunodisc electrophoresis of MIPS and of the protein fraction isolated from inl (89601). Antiserum was diluted with saline in 1 to 2 ratio.
1 and 2 : MIPS from wild type (30 µg protein)
3, 4 and 5: the protein fraction from inl (100 µg protein)
1 and 3 were stained directly with Coomassie brillant blue.
2, 4 and 5 were stained after immunoprecipitation.

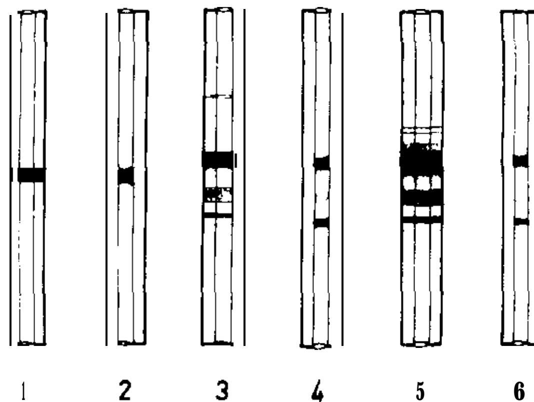

Figure 3. Immunodisc electrophoresis of MIPS isolated from different strains of N. crassa. The antiserum was diluted by saline in 1 to 2 ratio.
1 and 2: MIPS from wild type (30 µg protein)
3 and 4: MIPS from transformant (60 µg protein)
5 and 6: MIPS from spontaneous bock mutant (100 µg protein)
1, 3 and 5 were stained directly after run.
2, 4 and 6 were stained after immunoreaction.

type (specific activity = 4750 U/mg protein and K_M = 1.82 mM). The gel electrophoresic patterns were very similar to that of the protein fraction of inl mutant, i.e. both products, contained several proteins (Figure 3), but proteins that had nearly identical electrophoretic mobilities as that of MIPS from wild type gave a positive immune response. In addition, both protein fractions contained an additional immunological reacting component that ran faster than the wild type enzyme.

These results definitely support the idea that an inactive, defective protein homologous with MIPS of wild type is synthesized in the inositol requiring mutant allele 89601 and that the preparative procedure applied is suitable for its concentration and isolation. An enzyme species produced by the transformed strain is probably identical to that of the donor DNA strain, and to an enzyme species produced by the spontaneous revertant. (This work was supported by grants of the Hungarian Ministry of Health and the Hungarian Academy of Sciences.)- - - University Medical School of Debrecen, H-4012 Debrecen, POB 6, Hungary.