Fungal Genetics Reports

Volume 19 Article 1

Immunological studies with pyruvate dehydrogenase complex

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Recommended Citation
Harding, R. W., and R.P. Wagner (1972) "Immunological studies with pyruvate dehydrogenase complex," *Fungal Genetics Reports*: Vol. 19, Article 1. https://doi.org/10.4148/1941-4765.1859

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Abstract
Immunological studies with pyruvate dehydrogenase complex

Harding, R. W. and R. P. Wagner. Immunological studies with pyruvate dehydrogenase complex isolated from N. crassa.

The pyruvate dehydrogenase complex (PDC), which catalyzes the conversion of pyruvate to acetyl-CoA, has previously been purified from E. coli and mammals (Reed and Willmr 1966 Meth. Enzymol. 9:247) and from N. crassa (Harding et al. 1970 Arch. Biochem. Biophys. 138:653). The complex obtained from Neurospora was shown to have a diameter of 300-350 A, an S value of 85, and to resemble in many respects the particle isolated fmm mammals. The complex obtained from E. coli and mammals has been separated into three constitutive enzymes: pyruvate dehydrogenase, dihydrolipoyl transacetylase and dihydrolipoyl dehydrogenase (Reed and Willmr).

In the present study, purified PDC obtained from N. crassa was injected into a rabbit four times over a period of five weeks. The immune serum thus obtained was found to form a single line with purified PDC on an Ouchterlony plate. When purified PCC was incubated for 10 min. at 37°C with the immune serum, the overall activity for the conversion of pyruvate to acetyl-CoA, as measured by NAD reduction, was completely lost. There is still a 22% inhibition of this activity even when the immune serum is diluted by a factor of 40.

The dihydrolipoyl dehydrcgenare component of Neurospora PDC was assayed as described by Reed and Willmr. Some modification in their assay for the dihydrolipoyl transacetylase activity was necessary, and no success was obtained by using their assay for measurement of the pyruvate dehydrogenase component. The immune serum at its highest concentration does not inhibit to any great extent either dihydrolipoyl dehydrogenase or dihydrolipoyl transacetylase. If the Neurospora PDC has a similar structure to PDC isolated from other sources, then the dihydrolipoyl transacetylase probably is the core of the molecule and is surrounded by the other two enzyme components. It would thus be predicted that the transacetylase activity would not be inhibited by the immune serum. Perhaps potential sites of inhibition on the dihydrolipoyl dehydrogenase are also blocked by another component of the complex. Presumably the overall activity of the particle is inhibited because the antibody inhibits the activity of the proposed third component, namely, pyruvate dehydrogenase. However, until a satisfactory assay can be devised for this enzyme, this hypothesis cannot be tested. • • • Department of Zoology, University of Texas, Austin, Texas 78712 (Present address of RWH: Radiation Biology Laboratory, Smithsonian Institution, Rockville Maryland 20852.).