NOTES ON THE "IN VITRO" FORMATION OF NADPH — NITRATE REDUCTASE

NOTA SOBRE A FORMAÇÃO "IN VITRO" de NADPH — NITRATO REDUTASE.

* A. Nason
* K. Y. Lee
* S. S. Pan
* P. A. Ketchum
** A. Lamberti

* Departament of Biology, McCollum-Pratt Institute, The Johns Hopkins University, Beltimore, Md, U. S. A.

** Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo.

Bol. de Botânica **1**, *101-102 (1973)*

RESUMO

A presente nota refere-se à complementação "in vitro" obtida com extratos do mutante nit-1 de *Neurospora crassa* induzido por nitrato de sódio e diversas molibdo-enzimas acidificadas. Os resultados fortalecem a idéia de que a NADH-nitrato redutase consiste de pelo menos duas subunidades proteínicas diferentes: uma nitrato-induzível e outra, constitutiva, molibdênica.

The assimilatory nitrate reductase (NADPH: nitrate reductase, EC 1.6.6.2) from *Neurospora crassa* was first characterized by Nason & Evans (2), who purified the system about 70-fold and found it to be (a) an inducible, sulfhydryl-containing flavoprotein with FAD as the prostetic group, (b) relatively specific for NADPH as the electron donor, and (c) sensitive to a number of metalbinding agents.

That molybdenum is a metal constituent of the *Neurospora* enzyme (4, 5) was shown by (a) specific reactivation of the cyanide-dialysed enzyme upon addition of molybdenum, (b) proporcionality of the molybdenum content of enzyme fractions to the amount of nitrate reductase activity and (c) specific effect of molybdenum deficiency during growth of the mycelia, resulting in decreased nitrate reductase activity.

An assymilatory NADPH — nitrate reductase similar to the wild-type *Neurospora* enzyme was formed by *in vitro* complementation between extracts of nitrate induced *N. crassa* mutant nit-1 and (a) certain other nonallelic nitrate reductase mutants or uninduced wild-type (3), or (b) the acid-treated molybdo-enzymes xanthine oxidase and xanthine dehydrogenase and liver aldehyde oxidase from mammals and birds.

Some 20 non-molybdo-enzymes were inactive (1).

A similar *in vitro* complementation has now been attained using other acid-treated known molybdo-enzymes including:

(a) nitrogenase, or its molybdo-iron-protein, from *Clostridium pasteurianum, Azotobacter vinelandii,* and soybean nodule-bacteroids;
(b) bovine liver sulfite oxidase;
(c) respiratory nitrate reductase from *Escherichia coli,* and
(d) NADH — nitrate reductase from foxtail grass (*Setaria faberii*).

Several Mo-amino acid complexes, as possible catalytic models of nitrogenase, failed to complement. The results strengthen the view that NADPH-nitrate reductase consists of at least 2 dissimilar protein subunits: an nitrate — inducible subunit(s) involved in the early part of the electron transport chain, and a molybo-containing constitutive subunit(s) in the later part of the nitrate reductase chain.

The data also suggest the existence of a similar molybdo-protein subunit in the known molybdo-enzymes, whether from procaryotic or eucaryotic cells.

BIBLIOGRAFIA CITADA

KETCHUM, P. A., CAMBIER, H. Y., FRAZIER III, W. A., MADANSKY, C. H. and NASON, A. — 1970 — *In vitro* assembly of *Neurospora* assimilatory nitrate reductase from protein subunits of *Neurospora* mutant and the xanthine oxidizing or aldehyde oxidase systems of higher animals. PNAS, 66: 1016-1023.

NASON, A. and EVANS, H. J. — 1953 — Triphosphopyridine nucleotide-nitrate reductase in *Neurospora.* Jour. Biol. Chem. 202: 655-673.

NASON, A., ANTOINE, A. D., KETCHUM, P. A., FRAZIER III, W. A. and KEE, D. K. — 1970 — Formation of nitrate reductase by *in vitro* complementation between an extract of a *Neurospora* mutant and xanthine oxidase. PNAS, 65: 137-144.

NICHOLAS, D. J. D., NASON, A. and McELROY, W. D. — 1954 — Molybdenum and nitrate reductase. I. Effect of molybdenum deficiency on the *Neurospora* enzyme. Jour. Biol. Chem. 207: 341-351.

NICHOLAS, D. J. D. and NASON, A. — 1954 — Molybdenum and nitrate reductase. II Molybdenum as a constituent of nitrate reductase. Jour. Biol. Chem. 207: 353-360.