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An enzymatic defect in osmotic mutants of *Neurospora crassa*: lack of induction of NAD(P)ase activity during growth on casein.

Nicotinamide adenine dinucleotide (phosphate) glycohydrolase (NAD(P)ase; E.C. 3.2.2.6) activity in *Neurospora crassa* increases sharply during conidiogenesis and accumulates in mature wild-type conidia. In submerged vegetative cultures, NAD(P)ase is induced by deficiency of Zn^{2+} (Nason et al. 1951 J. Biol. Chem. 188:397-406) or by proteins as the sole source of carbon in the culture medium (Jorge and Terenzi 1984 J. Gen. Microbiol. 130:1563-1568). While screening for the production of exoenzymes in a "slime" strain of *N. crassa* (FGSC 1118: Pietro et al. 1989 J. Gen. Microbiol. 135 : in press) we observed "slime" spheroplasts did not produce NAD(P)ase under the same conditions that stimulated synthesis and secretion of the enzyme in the wild type (i.e. cultivation in the presence of protein). However, this defect was not related to the wall-less phenotype of "slime", because it was also observed for recombinants with osmotic (os-1) phenotype isolated from a cross of wild type and a "slime"-containing heterokaryon (FGSC 2713). On average, the level of induced extracellular NAD(P)ase activity in os-l recombinants was about 4% that of recombinants carrying the wild-type allele. Intracellular NAD(P)ase activity was also low.

The defect observed for os-l was also observed for other osmotic mutants, specifically os-1, os-5 and os-6 (Table 1), in which the induced extracellular NAD(P)ase activity of cultures incubated in the presence of casein ranged from zero (below the assay sensitivity) to 19% of the activity produced by wild-type cultures. For the os-4 mutant, the level of induced extracellular NAD(P)ase activity was about 50% of that of the wild type: therefore, we cannot conclusively classify this mutant as deficient in NAD(P)ase induction.

Table 1. NAD(P)ase activity in cultures of wild type, osmotic mutants and the *nada* mutant, incubated in the presence of casein.

Strain	NAD(P)ase specific activity	
	extracellular (units.mgDW-1)	intracellular (units.mg.prot.-1)
FGSC 424 (wild type)	0.84 ± 0.12	0.05 ± 0.03
FGSC 810 (os-1)	0.03 ± 0.02	0.02 ± 0.01
FGSC 2239 (os-2)	0.12 ± 0.04	undetected
FGSC 2430 (os-4)	0.40 ± 0.10	0.05 ± 0.01
FGSC 2243 (os-5)	0.06 ± 0.01	undetected
FGSC 3898 (os-6)	undetected	undetected
FGSC 2688 (nada)	undetected	undetected

Mycelia were grown with agitation in liquid Vogel's medium (2% sucrose) for 18 h at 30°C. At that time 100 mg samples were transferred to fresh medium (25 ml) supplemented with 1% cesein as sole source of carbon, and incubated for an additional 6 h period. NAD(P)ase activity was assayed in the culture filtrates and crude extracts according to Kaplan et al. (1951 J. Biol. Chem. 191:473-483). Specific NAD(P)ase activity of wild type cultures at zero time of induction was 0.01 and 0.07 in the culture filtrates and cell extracts, respectively. The results are the mean ± S.D. of three independent experiments.

The defect in NAD(P)ase synthesis of the osmotic mutants and the one present in the NAD(P)ase-deficient *nada* mutant were different. With one exception (os-1), which produced few conidia), the osmotic mutants exhibited wild-type levels of NAD(P)ase activity in aerial hyphae and conidia, while the enzyme was not detectable in the same structures formed by the *nada* mutant (Table 2). These results suggested that NAD(P)ase expression may be under the control of different regulatory circuits: one responding to developmental signals, and the other controlling the induction of NAD(P)ase in vegetative cells. Analogous suggestions have been made for other enzymes of *N. crassa* such as alkaline protease (Hanson and Marzluf 1975 Proc. Natl. Acad. Sci. USA 72:1240-1244), ribonuclease (Lindberg and Drucker 1984 J. Bacteriol. 157:380-384) and L-amino acid oxidase (Prade and Terenzi 1985 Arch. Microbiol. 143:37-41).

Table 2. NAD(P)ase activity in conidia and aerial hyphae of cultures of wild type, osmotic mutants and the nada mutant, cultivated in solid medium.

Strain	NAD (P)ase activity (units.mg protein-l)
FGSC 424 (wild type)	16.45
FGSC 810 (os-1)	2.11
FGSC 2239 (os-2)	9.64
FGSC 2430 (os-4)	12.57
FGSC 2243 (os-5)	28.65
FGSC 3898 (os-6)	18.87
FGSC 2688 (nada)	undetected

Is NAD(P)ase activity related to adaptation to osmotic stress? We investigated this possibility by cultivating the wild-type strain in liquid medium containing NaCl at concentrations which affect the growth of osmotic mutants. Under those conditions, extracellular NAD(P)ase activity increased 3.9-fold over control values. However, this increase was far below that induced by incubation in the presence of casein, which was 70 to 100-fold. Furthermore, although the effect of casein in inducing NAD(P)ase activity could in principle be attributed to an osmotic effect of the protein, it is difficult to understand why casein induction is abolished by glucose or some amino acids (Jorge and Terenzi 1984 J. Gen. Microbiol 130:1563-1568). Therefore, the question of the possible involvement of NAD(P)ase in adaptation to osmotic stress remains unanswered for the time being.

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