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Abstract

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Some observations indicate the presence of a resulatory mechanism in Neurospora crassa cells providing for an adaptation of the cells to an environmental stress. The influence of a thermal shock or incomplete inhibition of protein synthesis by cycloheximide induces formation of new polypeptides, as well as the synthesis of activation of some enzymes (Horowitz et al., 1970 J. Biol. Chem. 245 2784-2788; Perlman and Feldman, 1982 Mol. Cell Biol. 2: 1167-1173). The present paper demonstrates that NAD^+ kinase (ATP:NAD^+2,-phosphotransferase, EC 2.7.1.23), the enzyme catalyzing the formation of $NADP^+$, and thus controlling the $NADP^+(H)/NAD^+(H)$ ratio and playing a key role in coordination of the catabolic processes and biosynthetic reactions, may be a part of the adptational mechanisms.

The experiments were performed with three day-old surface cultures of N. crassa nada (100) a (FGSC #2696) (NAD^+/P^+/-glycohydrolase free mutant), which were grown on the cellophane film covering Vogel's agar medium (Vogel, 1964 Amer. Natur. 98: 435-446). To subject the cells to stress, the celophane films with hyphae were transferrred to a nutrient free agar and treated for three hours with some agents which could affect different metabolic processes (the control samples were incubated for the same period of time). Among these agents were osmotic shock after transfer of the cells under hypotonic conditions, inhibition of protein synthesis by cycloheximide (1 mg per Petri dish), impairment of the cell membrane structure by 50% ethanol or 0.1% Triton X-100, temperature, treatment - culture transferring from 28° (cultivation temperature) to 4° or 37°. NAD^+ synthesis catalyzed-by NAD^+ kinase was studied in a reaction mixture (1 ml total volume) containing 2 µM NAD^+, 3 µM ATP, 3 µM $MgCl_2$, dissolved in 0.05 M tris-HCl buffer, pH 8.3, and started by the addition of 0.05-0.1 ml enzyme preparation containing 150-300 µg protein. The quantity of $NADP^+$ was measured by the recycling assay, where $NADP^+$ dependent glucose-6-phosphate dehydrogenase reaction was conjugated with decoloration of thedox dye 2,6-dichlorophenolindophenol (Slater et al., 1964 Arch. Internat. Physiol. Biochem. 72: 427-447; Afanasieva et al., 1982 Arch. Microbiol. 133: 307-311).

The results summarized in Table I show that, except for temperature, shifts in the metabolism of Neurospora cells bring about a rise of NAD+ kinase activity. Furthermore, all agents that could cause sharp visible light which may lead to photodynamic damage of the cells, causes a two-fold increase of NAD^+ kinase activity in maturing conidia. The enzyme activity also rises during hydration of conidia. It is of interest that cycloheximide at a concentration completely blocking the protein synthesis increases the activity of NAD+ kinase. This may be evidence of activation of the enzyme molecules, rather than of their synthesis *de novo*. The absence of response, i.e. enzyme activation, in the case of the temperature treatment may be related to a slower and more gradual manner of its action, as compared with the other stress-inducing factors.

TABLE I

The specific activity of NAD^+ kinase from N. crassa cells subjected to the action of stress-inducing agents (nmol $NADP^+.hr^{-}.mg$ protein)

Treatment	Specific activity	
	nmol $NADP^+.hr^{-1}.mg$ protein^{-1}	Per cent
Control A (cells transferred to a nutrient free agar)	20.1+ - 1.0	100
Hypotonic shock	56.5+ - 9.5	281
Cycloheximide	68.1+ - 10.5	339
50% ethanol	53.0+ - 12.5	264
0.1% Triton x 100	43.7+ - 5.2	217
Control B (no transfer to a nutrient free agar)	25.7+ - 3.9	100
Temperature shift from 28° to 37°	26.2+ - 5.0	102
Temperature shift from 28° to 4°	27.8+ - 1.1	108

The effect of unfavorable environmental agents on microbial cells is known to disturb the balance between the rates of nutrient consumption and biosynthetic processes (Posmogova, 1974 Advances in Microbiol.[in Russian] 9: 84-85). The fact that NAD+ kinase activity in the Neurospora cells increases in response to the nonspecific action of very diverse stimuli, suggests that the enzyme plays some role in the re-establishment of the disturbed balance between anabolism and catabolism during adaptation. Our observations are paralleled by the experiments which demonstrated the involvement of NAD+ kinase in the adaptation of plant and animal organisms to the action of toxic compounds (Agosin et al., 1967 Can. J. Biochem. 45: 619-626; Harada *et al.*, 1980 J. Fac. Hokkaido University 59: 380-391).

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