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Metabolism of NAD^+ in Nuclei of *Saccharomyces cerevisiae* during Stimulation of Its Biosynthesis by Nicotinamide

T. G. Gulyamova*, D. M. Ruzieva, S. M. Nasmetova, M. R. Shakirzyanova, and A. M. Kerbalaeva

Institute of Microbiology, Uzbekistan Academy of Sciences, ul. Abdully Kodiry 7B, Tashkent, 700128 Uzbekistan; fax: (998-712) 417-129; E-mail: imbasru@uzsci.net

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Abstract—The activities of nuclear enzymes involved in NAD^+ metabolism in *Saccharomyces cerevisiae* strain 913a-1 and its mutant 110 previously selected as an NAD^+ producer were investigated. The presence of extracellular nicotinamide increased the total NAD^+ pool in the cells and increased [^{3}H]nicotinic acid incorporation; however, NAD^+ concentration in isolated nuclei decreased slightly. The stimulating effect of nicotinamide on intracellular synthesis of NAD^+ correlated with increases in ADP-ribosyl transferase, NAD^+-pyrophosphorylase, and NAD^+ase activities.

Key words: NAD^+, NAD^+-glycohydrolase, NAD^+-pyrophosphorylase, ADP-ribose, ADP-ribosyl transferase

NAD^+ is a substrate for reactions of poly- and mono-ADP-ribosylation of nuclear proteins and other macromolecules [1]. It is also involved in DNA replication, cell proliferation, regulation of gene expression, maintenance of chromatin structure, and protection of DNA against damages. The final stage of NAD^+ synthesis catalyzed by NAD^+-pyrophosphorylase occurs in the nucleus. Degradation of NAD^+ also involves nuclear enzymes NAD^+-glycohydrolase and ADP-ribosyl transferase. Thus, it is possible that changes of NAD^+ concentrations in the nucleus are a trigger mechanism regulating the activity of ADP-ribosylation, which utilizes NAD^+ as a substrate [2, 3]. Thus, it is of interest to investigate microorganisms that can overproduce NAD^+ from exogenous precursors, possibly due to altered regulation of its metabolism.

In the present study, we investigated changes in nuclear NAD^+ turnover during induction of its synthesis in *S. cerevisiae* strains previously selected as NAD^+ producers [4].

MATERIALS AND METHODS

Saccharomyces cerevisiae strain 913a-1, selected as an NAD^+ producer, was cultivated at 30-32°C for 72 h with shaking.

Mutants were obtained using nitroso-N-methylurea. Cells cultivated for two days were treated with 0.1, 0.3, and 0.5% mutagen solution for 30, 60, 90, and 120 min. Nitroso-N-methylurea treated cells were seeded onto an agarose medium and cultivated at 28-30°C for 7 days. Producers among these mutants were selected by determining NAD^+ content during submerged cell cultivation on the screening medium [4].

NAD^+ content was assayed spectrophotometrically in cell and nuclear extracts using yeast alcohol dehydrogenase (Reanal, Hungary) in the presence of ethanol [5].

Nuclei were isolated from yeast cells as described previously [6]. The purity and intactness of the isolated nuclei were evaluated using several parameters: 1) the ratio DNA/RNA/protein of 1 : 0.4 : 5.1 corresponding to characteristics of purified nuclear preparations; 2) the activity of NAD^+-pyrophosphorylase, which was about 20 μmol NAD^+ per min per mg nuclear protein or higher [7]; 3) light microscopy of stained preparations.

NAD^+ase activity was registered by the decrease of NAD^+ content during incubation of isolated nuclei [8]. Unreacted NAD^+ was measured by its reaction with 1 M KCN. One unit of NAD^+ase activity corresponded to the amount of enzyme catalyzing cleavage of 0.1 nmol NAD^+ at 37°C during 20 min.

NAD^+-pyrophosphorylase activity was determined as described previously [9]. Briefly, aliquots of freshly isolated nuclei were incubated at 37°C for 20 min in medium

* To whom correspondence should be addressed.

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(total volume 0.8 ml) containing 0.3 ml 0.25 M glycylglycine-KOH buffer, pH 7.4, 0.5 μM ATP, 0.3 μM nicotinamide mononucleotide, 0.3 μM nicotinamide, 1.5 μM $MgCl_2$, and 1 mg of nuclear protein. One unit of NAD^+-pyrophosphorylase activity corresponded to the amount of enzyme catalyzing cleavage of 1 μmol NAD^+ during 20 min.

For the determination of basal ADP-ribosyl transferase activity, the isolated cell nuclei were resuspended in 5 ml of 0.1 M Tris-HCl buffer, pH 8.0, containing 40 mM KCl, 10 mM $MgCl_2$, 2 mM dithiothreitol, and 0.08 MBq/ml [^{14}C]NAD^+. The mixture was incubated at 37°C for 20 min, and the reaction was stopped by adding 3 ml of 20% trichloroacetic acid. The acid-insoluble material retained by Millipore filters was washed with trichloroacetic acid, the filters were dried, and the radioactivity entrapped into them was counted using a scintillation counter. The total enzyme activity was determined under similar conditions but in the presence of 0.5% Triton X-100 (v/v) and 10 U/ml DNase I. Specific radioactivity of [^{14}C]NAD^+ was reduced to 1.25 Ci/mol. ADP-ribosyl transferase activity was expressed as counts per min of [^{14}C]NAD^+ bound to the acid-insoluble material per mg nuclear protein. Incorporation of [^{3}H]nicotinic acid was registered during 60 min at 1 mM concentration and specific radioactivity of 0.85 Ci/mol.

For the identification of ADP-ribose, the acid-insoluble fraction obtained after sedimentation of nuclei labeled with [^{14}C]adenine-NAD^+ was dissolved in 0.1 M NaOH and neutralized by adding 0.1 M HCl and 0.5 M Tris-HCl buffer, pH 7.6, containing 200 mM $MgCl_2$. The mixture was incubated at 37°C for 30 min in the presence of one of the following enzymes (100 U/ml): nuclease from *Micrococcus* (grade VI) (Sigma, USA), snake venom phosphodiesterase, or proteinase K. After termination of the reactions, radioactivity was determined using the liquid scintillation counter [10].

RESULTS AND DISCUSSION

Cultivation of the initial strain and mutant 110 of *S. cerevisiae* in the medium containing 1 mM nicotinamide was accompanied by marked increase of intracellular NAD^+ concentration in the steady state growth phase, whereas in the nuclei there was some decrease of the coenzyme concentration (Table 1).

Increase of proliferative activity of normal and transformed cells [11, 12] and DNA repair and replication [13, 14] are accompanied by the exhaustion of the total pool of pyridine dinucleotides. The decrease of NAD^+ level induced by DNA damaging agents does not represent the metabolic consequence of inhibition of synthesis or activation of NAD^+ase. It occurs due to the activation of ADP-ribosyl transferase in the nucleus. It is generally accepted that synthesis of poly(ADP)-ribose is responsi-

Table 1. The effect of exogenous nicotinamide (1 mM) on total and nuclear content of NAD^+ and the activity of nuclear enzymes of NAD^+ metabolism

S. cerevisiae strain	NAD^+ content, mmol per mg protein		Enzyme activity, U/mg protein		[^{3}H]nicotinic acid incorporation (cpm/mg protein) × 10^3
	total	nuclear	NAD^+ase	NAD^+-pyrophosphorylase	
913a-1					
control	0.021	0.018	0.64	3.8	190
+ nicotinamide	0.037	0.012	0.96	4.6	160
Mutant 110					
control	0.042	0.016	0.72	2.8	170
+ nicotinamide	0.089	0.013	0.97	6.4	340

Table 2. Basal and total ADP-ribosyl transferase activity during induction of NAD^+ synthesis by nicotinamide

S. cerevisiae strain	ADP-ribosyl transferase activity (cpm [^{14}C]adenine per mg protein) × 10^3			
	control		+ nicotinamide	
	basal	total	basal	total
913a-1	70	101	121	148
Mutant 110	84	115	100	110

Table 3. Identification of [^{14}C]ADP-ribose in nuclei of *S. cerevisiae* cells

Sample	[^{14}C]ADP-ribose, cpm/mg protein	Degradation, %
Control	4375	0
Sequential treatment with:		
DNase	3692	12.8
proteinase K	3825	29.6
phosphodiesterase	48	98.9

ble for the decrease in NAD^+ [12]. In HeLa cells, a half-turnover period of NAD^+ is about 1 h and degradation of 95% of the coenzyme in the nucleus involves ADP-ribosyl transferase [14]. Consequently, it is reasonable to suggest that changes in biosynthesis of ADP-ribose should correlate with the changes in NAD^+ concentration. We found that the decrease of nuclear concentration of NAD^+ during stimulation of its synthesis in cells of the initial and mutant 110 strains of *S. cerevisiae* by nicotinamide correlated with the increase of NAD^+-pyrophosphorylase and NAD^+ase activities in the nuclei (Table 1).

In addition to the increase of intracellular concentration of NAD^+, we also detected an increase of basal and total ADP-ribosyl transferase activity determined by the incorporation of [^{14}C]adenine from NAD^+ into native and DNase treated nuclei of *S. cerevisiae* cells (Table 2).

Table 3 shows that [^{14}C]adenine-NAD^+ labeled material of yeast nuclei was almost completely degraded by enzymatic hydrolysis with snake venom phosphodiesterase, whereas DNase and proteinase were ineffective. This suggests that the labeled product is ADP-ribose bound to nuclear proteins.

Thus, the induction of NAD^+ synthesis in yeast cell producers is characterized by the activation of nuclear metabolism of this coenzyme. The activation of NAD^+-pyrophosphorylase increases the rate of NAD^+ biosynthesis, whereas NAD^+ase and ADP-ribosyl transferase utilize this coenzyme. The latter is accompanied by the increased incorporation of labeled ADP-ribose. This suggests an increase of protein modification in the nucleus that may reflect structure–function changes of the chromatin.

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