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# The NAD$^+$ Precursors, Nicotinic Acid and Nicotinamide Upregulate Glyceraldehyde-3-Phosphate Dehydrogenase and Glucose-6-Phosphate Dehydrogenase mRNA in Jurkat Cells

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**To better understand the role of nicotinic acid and nicotinamide in the regulation of the oxidative stress response, we measured the levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and glucose-6-phosphate dehydrogenase (G6PD) mRNA in Jurkat cells treated with these NAD$^+$ precursors. We used a modified nonradioactive Northern blot method and detected the mRNA using 18-mer digoxigenin (DIG)-labeled oligonucleotides as probes. We observed increased levels of the mRNAs for the two enzymes in treated cells. Our findings suggest that the NAD$^+$ precursors may protect against oxidative stress and DNA damage by up-regulating the stress response genes GAPDH and G6PD.** 

***Key Words:*** **oxidative stress; glyceraldehyde-3-phosphate dehydrogenase; glucose-6-phosphate dehydrogenase; nicotinic acid; nicotinamide.**

Oxidative stress has been reported to be associated with numerous degenerative conditions, including neurodegenerative diseases, heart disease, cancer and aging (1). The glycolytic enzymes, glucose-6-phosphate dehydrogenase (G6PD) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), appear to protect cells against oxidative stress (2–4). Presumably, this protective action involves generation of the reduced pyridine nucleotides, NADPH and NADH, from NAD$^+$ (Fig. 1). The generation of these reducing equivalents depends upon an adequate supply of NAD$^+$. Intracellular levels of NAD$^+$ can be effectively increased by the addition of the NAD$^+$ precursors, nicotinic acid (NA) and nicotinamide (NAM) (5, 6). We hypothesized that treatment of cells with these NAD$^+$ precursors may upregulate G6PD and GAPDH transcripts. We now report that the mRNA levels of both genes are significantly elevated by treatment of cells with NA and NAM. This finding may indicate new approaches to the treatment of diseases associated with oxidative stress.

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## MATERIALS AND METHODS

*Chemicals.* NA and NAM, and all general chemicals were purchased from Sigma (St. Louis, MO). Agarose, cesium chloride, and sodium dodecyl sulfate (SDS) were obtained from Gibco BRL Life Technologies (Grand Island, NY). DIG Easy Hyb, DIG Wash and Block Buffer Set, disodium 3-4[4-methoxyspiro(1,2-dioxetane-3,2′-(5′-chloro)tricyclo[3.3.1.1$^{3,7}$]decan)-4-yl]phenyl phosphate) (CSPD), anti-DIG Fab fragments, DIG-labeled actin RNA probe and positively charged nylon membranes were purchased from Boehringer Mannheim (Indianapolis, IN). Digoxigenin (DIG)-labeled GAPDH and DIG-labeled G6PD antisense oligonucleotide probes were synthesized by Synthegen (Houston, TX).

*Preparation of cultured cells.* The Jurkat cell line was derived from a human T-cell lymphoma [American Type Culture Collection (ATCC), Bethesda, MD: ATCC #TIB 152]. Cells were maintained in RPMI 1640 medium from Irvine Scientific (Santa Ana, CA). The medium was supplemented with 10% fetal calf serum (Omega Scientific, Inc., Tarzana, CA), 1% penn/strep, 20 mM HEPES Buffer and 1% L-glutamine (Gibco). Cultures were maintained at 37°C in a humidified atmosphere of 95% air and 5% $CO_2$. To prepare treated cells, Jurkat cells (in complete RPMI 1640 medium), were exposed to either 10 mM NA or 10 mM NAM for 24 hours. Cultures without the treatment were used as controls.

*RNA isolation.* NA and NAM-exposed and control cultures (each containing $2 \times 10^8$ Jurkat cells) were lysed in guanidinium isocyanate and total RNA was collected by ultracentrifugation through a cesium chloride step gradient (7). RNA aliquots, containing 20 $\mu$g RNA, were separated by electrophoresis in a 1% agarose-formaldehyde gel.

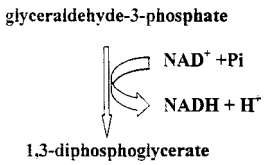
*Preparation of probes.* Antisense oligonucleotide probes were designed for GAPDH (5′-GGTGGAGGAGTGGGTGTC-3′) (GenBank AC#X01677) and G6PD (5′-CCTCTCATTCTCCACATA-3′) (Gen-



0006-291X/99 $30.00

A

**Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the following reaction:**




B

**Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the following reaction:**

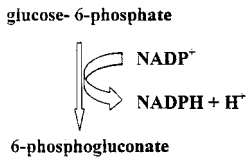



**FIG. 1.** The reactions catalyzed by GAPDH and G6PD generating NADH and NADPH, respectively.

Bank AC#X03674) using Eugene software (Daniben System, Inc.). Both probes were synthesized and labeled at the 5′ end with digoxigenin (DIG) by Synthegen (Houston, TX).

*Nonradioactive Northern blot hybridization.* RNA was transferred from the gel to positively charged nylon membranes (Boehringer Mannheim) by capillary transfer with 20× SSC overnight. RNAs are crosslinked to membranes by exposure to 120,000 $\mu$J UV light using a UV Stratalinker (Stratagene). Mock hybridization was performed to determine appropriate probe concentration. The blot was prehybridized for 1 hour in 30 ml of prewarmed hybridization buffer (DIG-Easy Hyb, Boehringer Mannheim). The prehybridization solution was then replaced with 30 ml of prewarmed hybridization buffer containing either 30ng/ml DIG-end labeled $\beta$-actin probe, 3 nmol/ml DIG-end labeled GAPDH probe, or 3nmol/ml DIG-end labeled G6PD probe and hybridized overnight. The blot was washed in washing buffer (from the DIG Wash and Block Buffer Set) for 10 minutes. The membrane was incubated in blocking solution (Boehringer Mannheim), followed by blocking solution containing the anti-DIG-AP conjugate (1:10,000 dilution), and then equilibrated in detection buffer containing the chemiluminescent substrate CSPD (Boehringer-Mannheim). The membrane was then exposed to X-ray film at room temperature for 4 hours for the actin probe, and for 2 hours at room temperature for the GAPDH and G6PD probes.

*Digital representation of relative mRNA transcripts.* A digital image was made from each X-ray film obtained after treatment of nylon membranes with DIG-labeled antisense probes against GAPDH, G6PD and $\beta$-actin followed by anti-DIG antibodies and chemiluminescent detection, as described above. The digital image was imported into the hard drive of an NEC computer using a framegrabber. To control for different backgrounds in the X-ray films, a density profile was obtained for each lane using the line density function of the Image Pro-Plus software, version 3.0 (Media Cybernetics, Silver Spring, MD). A total of 15 gray level measurements across each of the dense bands were made at 1 mm intervals (measured on the computer monitor screen). All density profiles obtained from the same nylon membrane were then normalized so that each scan began at the same density value. Normalized density profiles for all lanes on the same nylon membrane are then plotted together to reveal alterations in RNA levels under different experimental situations.

## RESULTS

The effect of NA and NAM on the mRNA levels of the oxidative stress response genes, GAPDH and G6PD, was determined using Jurkat cells, a human lymphoma cell line. Total RNA was isolated from Jurkat cells after exposure to 10 mM NA or 10 mM NAM for 24 hours. These concentrations were determined to be non-toxic to cells and did not induce either apoptosis or necrosis (unpublished data). We then determined the levels of GAPDH, G6PD, and $\beta$-actin mRNAs, using a non-radioactive Northern blot analysis method (Fig. 2).

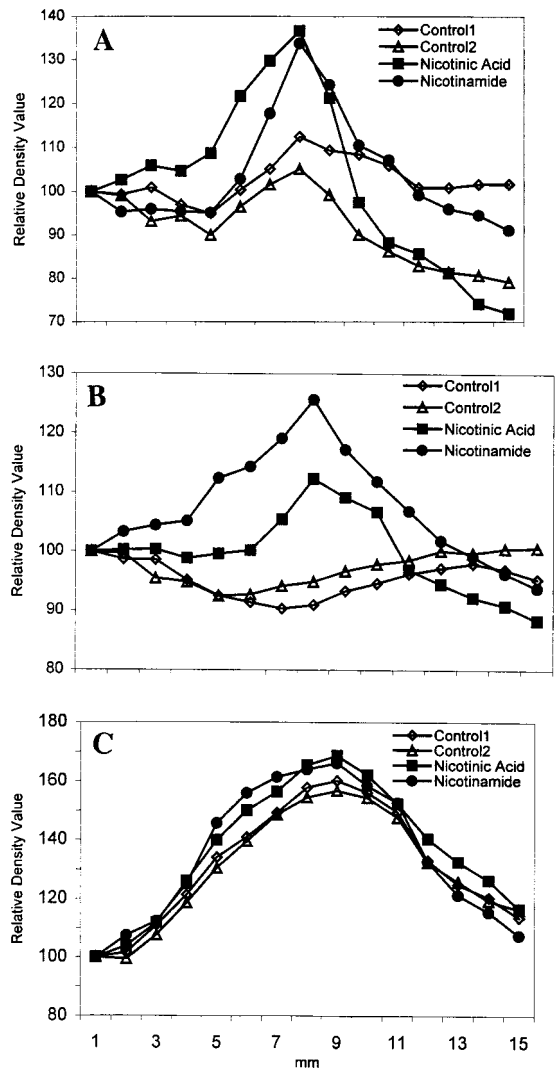



**FIG. 2.** Analysis of mRNA expression of GAPDH (A), G6PD (B) and $\beta$-actin (C) in control (untreated) Jurkat cells and cells treated with NA and NAM. Nonradioactive Northern blots were performed as described in Materials and Methods. The relative densities of the bands were analyzed using computerized image analysis. The x-axis represents the points (in millimeters) that were measured across a give band on the computer monitor.

GAPDH mRNA levels of Jurkat cells exposed to NA or NAM for 24 hours were increased compared to GAPDH mRNA levels in control cells without pretreatment. Fig. 2A shows the analysis of relative densities of the bands using digital image analysis. The graph indicates a notable increase in GAPDH mRNA in cells treated with NA and NAM compared to controls. Whereas the NA-and NAM-treated cells gave density values 35-38% above background, the untreated controls gave values only 4-13% above background. This indicates at least a 2.7-fold increase in GAPDH mRNA upon treatment with the $NAD^+$ precursors.

We then examined the expression of the G6PD gene, another stress response gene, in Jurkat cells treated with either NA or NAM (Fig. 2B). Transcription of this gene was undetectable in untreated control cells. However, the G6PD mRNA level was notably elevated in cells treated with either NA or NAM.

As a control for loading gels, we also measured $\beta$-actin mRNA levels in Jurkat cells with and without treatment by NA or NAM. Similar $\beta$-actin mRNA levels were observed in both treated and untreated cells (Fig. 2C). Spectrophotometrically, there also did not appear to be a general increase in overall mRNA levels after treatment with NA and NAM. Therefore, the increased levels of GAPDH and G6PD mRNA after treatment with $NAD^+$ precursors likely reflects a specific effect on either gene expression or mRNA stability.

## DISCUSSION

We have shown in the present study that the $NAD^+$ precursors, NA and NAM, upregulate the mRNA levels of two genes, GAPDH and G6PD, both associated with protection of cells against oxidative stress (2–4). These two genes are often considered to be "housekeeping" genes as their gene products are glycolytic enzymes. However GAPDH produces NADH from $NAD^+$, and G6PD produces NADPH from $NADP^+$ (through the pentose phosphate shunt), so that they alter the redox state of the cell in favor of reducing equivalents (Figure 1). NADPH, for example, is essential for the synthesis of glutathione, a major nonprotein thiol in the cell, by glutathione reductase. Therefore, "defense against oxidative stress" may be considered a "housekeeping" function of both of these enzymes.

NAM, as a precursor for $NAD^+$, prevents apoptosis in the brain induced by oxidative stress (6). Results from our laboratory have shown that NA and NAM protect cells against apoptosis induced by the natural detergent, sodium deoxycholate (NaDOC) (unpublished data). NaDOC induces oxidative stress, as evidenced by the introduction of oxidative DNA damage (8) and also by the activation of NF-$\kappa$B (9). Thus, the upregulation of GAPDH and G6PD mRNA levels by NA and NAM may help defend cells against oxidative damage leading to apoptosis.

Although the expression of housekeeping genes is usually kept at a relatively stable level, it has been reported that GAPDH mRNA levels can be hormonally and nutritionally regulated (10). Our results indicate that GAPDH levels can also be regulated by treatment of cells with $NAD^+$ precursors. [Note: caution should be taken if using GAPDH as an RNA control for loading gels].

NA and NAM can be converted to $NAD^+$ and $NADP^+$ in cells (5). While $NAD^+$ has a role in maintenance of the redox state of cells and ATP production, a major role of $NAD^+$ is as a substrate for poly(ADP-ribose) polymerase (PARP), an enzyme involved in DNA base excision repair (11). The synthesis of ADP-ribose polymers occurs as a rapid cellular response to DNA damage (5, 12). The cellular content of $NAD^+$ is crucial in determining the response of target cells to DNA damage. Cells can be readily depleted of $NAD^+$ during growth if there is a deficiency of NA or NAM. Even a mild deficiency of dietary niacin reduces $NAD^+$ and poly(ADP-ribose) levels in rat liver (13). Depletion of $NAD^+$ causes a decrease in PARP activity and reduced DNA repair of single-strand breaks (14). DNA repair in cells with DNA damage is stimulated by NAM (15). Excessive DNA damage may be a signal for cells to undergo apoptosis. Therefore, NA and NAM may exert their protective effect against apoptosis as precursors of $NAD^+$, a substrate for PARP activity to promote repair of DNA damage.

Another mechanism for increasing in DNA repair is through up-regulation of DNA repair enzymes. Relevant to the present study, NA and NAM upregulate GAPDH mRNA levels. A novel function for GAPDH in DNA repair has been recently reported (16). In its tetramer configuration, GAPDH functions as a glycolytic enzyme and produces NADH from $NAD^+$; however, in its monomer state, GAPDH is a DNA uracil glycosylase, and functions in DNA repair (16).

In conclusion, our findings suggest that NA and NAM may protect against oxidative stress and DNA damage by upregulating the mRNA levels of stress response genes, GAPDH and G6PD. In addition to their roles in energy metabolism, these "housekeeping" gene products catalyze formation of NADH and NADPH respectively, the reduced forms of the pyridine nucleotides that serve to protect cells against oxidative stress. The increase in GAPDH expression could also contribute to DNA repair through its activity as a DNA uracil glycosylase. NA and NAM are $NAD^+$ precursors and provide the substrate for PARP, a stress-response enzyme involved in DNA repair. Since NA and NAM can serve as dietary supplements, they may provide protection against oxidative stress and DNA damage associated with neurodegenerative diseases, heart disease, cancer and aging.

## ACKNOWLEDGMENTS

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