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Glyceraldehyde-3-phosphate dehydrogenase: A universal internal control for Western blots in prokaryotic and eukaryotic cells

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ABSTRACT

In the current study, we examined the expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein in a number of organisms and the stability of GAPDH under various conditions. Our results revealed that GAPDH is present in multiple *Escherichia coli* strains, the yeast strain GS115, *Caenorhabditis elegans*, rat PC12 cells, and both mouse and rat brain. Furthermore, GAPDH was stably expressed under different concentrations of inducer and at different times of induction in *E. coli* (BL21) cells and yeast GS115 cells. Stable expression of GAPDH protein was also observed in *C. elegans* and PC12 cells that were treated with different concentrations of paraquat or sodium sulfite, respectively. In addition, we were able to detect and identify the endogenous gapA protein in *E. coli* via immunoprecipitation and MALDI-TOF-MS analysis. Endogenous gapA protein and exogenously expressed (subcloned) GAPDH proteins were detected in *E. coli* BL21 but not for gapC. With the exception of gapC in *E. coli,* the various isoforms of GAPDH possessed enzymatic activity. Finally, sequence analysis revealed that the GAPDH proteins were 76% identical, with the exception of *E. coli* gapC. Taken together, our results indicate that GAPDH could be universally used as an internal control for the Western blot analysis of prokaryotic and eukaryotic samples.

Introduction

The glyceraldehyde-3-phosphate dehydrogenase (GAPDH)[2] superfamily is encoded by different genes in different organisms such as gapA, gapB, and gapC in bacteria [1–4]; TDH-1, TDH-2, and TDH-3 in yeast [5,6]; gpd-1, gpd-2, gpd-3, and gpd-4 in *Caenorhabditis elegans* [7,8]; and G3PDH in rats [9]. GAPDH is widely distributed from prokaryotes to eukaryotes and is a typical housekeeping gene. Previous reports have shown that GAPDH is a vital glycolytic enzyme that is mainly involved in catalyzing the conversion of D-glyceraldehyde-3-phosphate to glycerate-1,3-bisphosphate. In addition to its glycolytic function, GAPDH has also been shown to regulate gene transcription under different metabolic states [10]. Moreover, GAPDH has been shown to initiate apoptosis in response to cell stress through nitric oxide (NO)-mediated S-nitrosylation [11,12]. Tisdale and Artalejo also reported that GAPDH is involved in the transport of vesicles from the endoplasmic reticulum (ER) to the Golgi apparatus [13]. Taken together, these studies reveal the complex functions of GAPDH.

As a housekeeping gene/protein, GAPDH has been widely used as a control in Western blot experiments [14] and reverse transcription polymerase chain reaction (RT–PCR) assays [15–17]. A previous study by our lab suggested that the endogenously expressed GAPDH protein might be useful as an internal control for the *Escherichia coli* strain BL21(DE3) in Western blot experiments [18]. In the current study, we used the mouse-derived anti-GAPDH monoclonal antibody to systematically identify the endogenously expressed GAPDH proteins that are present in prokaryotes and eukaryotes. We found that the mouse-derived anti-GAPDH monoclonal antibody employed in this study could recognize most of the GAPDH proteins that are present in different species, including gapA in *E. coli;* gpd-1, gpd-2, gpd-3, and gpd-4 in *C. elegans;* gap-3 in the yeast strain GS115; and G3PDH in rat PC12 cells. However, gapC in *E. coli* could not be detected with the mouse-derived anti-GAPDH

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[2] *Abbreviations used:* GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NO, nitric oxide; ER, endoplasmic reticulum; RT–PCR, reverse transcription polymerase chain reaction; LB, Luria–Bertani; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; IPTG, isopropyl β-d-1-thiogalactopyranoside; PBS, phosphate-buffered saline; MALDI–TOF–MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TBST, in Tris-buffered saline with Tween 20.

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monoclonal antibody. Our data demonstrate that GAPDH can be universally used as an internal loading control for Western blot analysis in both prokaryotic and eukaryotic organisms.

Materials and methods

Preparation of GAPDH proteins from different species

The GAPDH proteins in the HB101, BL21, OP50, and DH5α strains (Invitrogen, Carlsbad, CA, USA) of *E. coli*; the *Pichia pastoris* GS115 strain (Stratagene, La Jolla, CA, USA) of yeast; the N2 strain (Caenorhabditis Genetics Center [CGC], St. Paul, MN, USA) of *C. elegans*; rat PC12 cells (American Type Culture Collection [ATCC], Manassas, VA, USA); C57 mice (18–30 g, Vital River Laboratories, China); and Wistar rats (280–320 g, Vital River Laboratories) were prepared according to the following procedures.

Preparation of GAPDH protein from E. coli

E. coli cells (50 μl) were inoculated in 5 ml of Luria–Bertani (LB) medium. Cells were cultured with shaking (220 rpm) at 37 °C until the logarithmic growth phase. To prepare total protein samples under normal growth conditions, the bacterial cells were harvested and treated with a cell lysis solution (50 mM Tris–HCl [pH 6.8], 15 mM NaCl, 5 mM ethylenediaminetetraacetic acid [EDTA], 0.5% Nonidet P-40, and 1 mM phenylmethanesulfonyl fluoride [PMSF]). Additional cultures were treated with varying concentrations of isopropyl β-D-1-thiogalactopyranoside (IPTG, 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mM, Sigma–Aldrich, St. Louis, MO, USA) for 5 h to induce the expression of proteins. Different induction times (0, 1, 2, 3, 4, and 5 h) were also used at a concentration of 1.0 mM IPTG. All of the samples were harvested and treated with a cell lysis solution (see below) when an OD_{600} of 0.6 to 1.0 was reached.

Preparation of GAPDH protein from yeast

Yeast GS115 cells (100 μl) were inoculated into 5 ml of LB medium and cultured with shaking (220 rpm) at 30 °C until the logarithmic growth phase. To prepare total protein samples under normal growth conditions, the yeast cells were harvested and treated with a cell lysis solution. Additional cultures were treated with varying concentrations of methanol (0, 6.25, 12.5, 18.75, 25, and 50 mM) for 5 days to induce the expression of proteins. Different induction times (0, 1, 2, 3, 4, and 5 days) were also used with a concentration of 50 mM methanol. All of the samples were harvested and treated with cell lysis solution when an OD_{600} of 0.6 to 1.0 was reached.

Preparation of GAPDH protein from C. elegans

To prepare total proteins under normal growth conditions, the *C. elegans* N2 strain was fed with the *E. coli* bacterial strain OP50 on nematode growth medium (NGM) agar plates and was cultured in a 20 °C incubator for 48 h. The worms were subsequently synchronized with 0.5 M NaOH containing 0.5% NaClO. The L1 stage worms were then harvested and treated with different concentrations of paraquat (0, 0.1, 0.2, 0.3, 0.4, and 0.5 mM, Sigma–Aldrich). Finally, total protein samples were prepared with a cell lysis solution.

Preparation of GAPDH protein from rat PC12 cells

Rat PC12 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma–Aldrich) supplemented with 10% horse serum (HS, Gibco BRL, UK) and 5% fetal bovine serum (FBS, Gibco BRL) in a 37 °C incubator with 5% CO_2 to 80% to 90% confluency. Hypoxia was induced with different concentrations of sodium sulfite (0, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/ml) according to our previous work [19]. The cells were harvested, and total protein samples were prepared with a cell lysis solution.

Preparation of GAPDH protein from C57 mice and Wistar rats

C57 mice and Wistar rats were sacrificed, and the brain tissues were prepared according to a previous report [20]. The brain tissues were pestled and lysed by ultrasonication in a cell lysis solution to prepare total protein samples.

Immunoprecipitation and MALDI–TOF–MS analysis

E. coli HB101 cells were cultured to the logarithmic growth phase, harvested, and treated with a nondenaturing lysis buffer (20 mM Tris–HCl [pH 8.0], 137 mM NaCl, 10% glycerol, 1% Nonidet P-40, 2 mM EDTA, 2.5 mM lysozyme, and 50 μg/ml PMSF). The resulting lysates were centrifuged at 4 °C and 12,000 rpm for 10 min, and the supernatants were retained on ice for 1 h. Next, 100 μl of an A/G agarose bead slurry was added, and the solutions were incubated at 4 °C for 30 min with gentle agitation. The samples were then centrifuged at 4 °C and 12,000 rpm for 10 min. The bead pellets were discarded, and the supernatants were reserved for immunoprecipitation.

Mouse-derived anti-GAPDH monoclonal antibody (5 μl) was added to the supernatants (total protein was 100 μg), and the samples were incubated at 4 °C overnight. Next, 40 μl of an A/G agarose bead slurry was added, and the samples were incubated at 4 °C overnight. The next day, the samples were centrifuged and the supernatants were discarded. The remaining bead pellets were washed three times with ice-cold phosphate-buffered saline (PBS, 10 min each time). Following the washes, 30 μl of 5× loading buffer was added to each pellet, and the samples were boiled at 100 °C for 10 min. The samples were then fractionated by electrophoresis through 15% polyacrylamide gels. The protein bands corresponding to the size of GAPDH protein were excised and identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI–TOF–MS). The Mascot peptide mass fingerprint search program (http://www.matrixscience.com/search_form_select.html) was used to match peptide masses with proteins in the GenBank protein database (http://www.ncbi.nlm.nih.gov/protein).

Construction of prokaryotic expression vectors

Total RNA was extracted from the *E. coli* strains HB101, BL21, OP50, and DH5α; the yeast strain GS115; the *C. elegans* N2 strain; and the rat PC12 cells by a Total RNA Kit II (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. Next, reverse transcription was performed using a Reverse Transcription System (Sigma–Aldrich). Subsequently, the genes encoding the various GAPDH proteins present in each organism (Table 1) were amplified by PCR. The amplicons were then subcloned into the prokaryotic expression vector pET28b (Table 2), and the presence of each gene was confirmed by direct DNA sequencing.

Determination of expression levels of GAPDH proteins

The plasmids were transformed into *E. coli* BL21(DE3) cells, and the resulting plates were incubated in a 37 °C incubator for approximately 10–12 h. Clones harboring each plasmid were inoculated in 5 ml of LB medium containing kanamycin (100 μg/ml, Sigma–Aldrich). Cultures were grown with shaking (220 rpm) at 37 °C until the logarithmic growth phase. Next, 150-ml aliquots