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IDENTIFICATION OF THE ATP-BINDING HEAT-INDUCIBLE PROTEIN OF MR=37,000 AS GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

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SUMMARY: We previously found a novel ATP-binding heat-inducible protein of Mr=37,000 in BALB/c 3T3 cells. Here, we found that the peptide mapping of this 37-kDa protein was similar to that of rabbit glyceraldehyde-3-phosphate dehydrogenase. Therefore, we biochemically compared the 37-kDa protein with a product translated from mRNA which was hybrid-selected using a cDNA for encoding chick glyceraldehyde-3-phosphate dehydrogenase and found that these two proteins were very similar. Northern blotting analysis using its cDNA as a probe revealed that glyceraldehyde-3-phosphate dehydrogenase was a heat-inducible protein in BALB/c 3T3 cells and that it was induced by stresses including treatment with α, α'-dipyridyl.

Heat shock and other types of stress induce a set of proteins termed heat shock proteins or stress proteins (1). Some stress proteins are expressed abundantly under normal growth condition and function in cellular metabolism (2). Recently, we found a novel stress protein of Mr=37,000 (3). The 37-kDa protein can bind to ATP and is induced two- to three-fold by heat-shock of BALB/c 3T3 cells and over five-fold by an iron chelating reagent which does not induce other stress proteins. Synthesis of this protein also increased after neoplastic transformation. We have now identified the 37-kDa protein to be the glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

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Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HSP, heat shock protein; HSE, heat shock element.

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MATERIALS AND METHODS

Materials--- BALB/c 3T3 A31 clone was obtained from The American Type Culture Collection. ATP-agarose (linked through C-8) and rabbit GAPDH were purchased from Pharmacia and Sigma, respectively. Chick GAPDH cDNA (1.2 kb PstI fragment of pGAD-28, see ref. 4) was a kind gift from Dr. S. Hatanaka (Institute for Virus Research, Kyoto University, Kyoto, Japan). Human heat shock protein 70 (HSP70) cDNA (2.3 kb HindIII/BamHI fragment of pH2.3, see ref. 5) was kindly donated from Dr. R.I. Morimoto (Northwestern University, Chicago). Mouse β-actin cDNA (0.75 kb EcoRI/BamHI fragment of pMAβ-3'ut, see ref. 6) and mouse 28S rRNA specific probe (7.3 kb EcoRI fragment of pHr14E3) were obtained from Dr. S. Sakiyama (Chiba Cancer Center Research Institute, Chiba, Japan) and from JCRB gene bank (Tokyo, Japan), respectively. The *in vitro* translation system was obtained from New England Nuclear. Other reagents purchased from Wako Pure Chemical (Osaka, Japan) and Nacalai Tesque (Kyoto, Japan) were of the highest purity available from these companies.

Cell Culture and Treatment--- BALB/c 3T3 cells were cultured in Dulbecco's Modified Eagle's medium supplemented with 10% calf serum (Flow Labora-tories). Cells were treated with 0.3 mM α, α'-dipyridyl for 8 h, or 100 ng/ml phorbol myristate acetate for 1.5 h before harvesting (3).

Gel Electrophoresis, and Peptide Mapping--- Cells were lysed using 1% Nonidet P-40 as described previously (3). Cell lysates were applied to two dimensional gel electrophoresis consisting of non-equilibrium pH gradient gel as the first dimension and SDS-10% polyacrylamide gel electro-phoresis as the second (3). The 37-kDa bands were excised and peptide mapping was performed as previously described by Cleveland et al. (7) using V8 protease. Peptides were detected by silver-staining methods (8).

Hybrid-Selection and In Vitro Translation--- Hybrid-selection was performed according to the method of Parnes et al. (9). Briefly, a 3.3 ug cDNA fragment of chick GAPDH was incubated in 50 ul of 0.2 M NaOH for 30 min at room temperature and neutralized by adding 500 ul 6x SSC. The cDNA was blotted onto a nitrocellulose filter which was then washed with 6x SSC and baked at 80 °C for 3 h. The poly A^+-RNA was extracted from BALB/c 3T3 cells, sus-pended in 500 ul hybridization solution (20 mM Pipes, pH 6.4, 65% formamide, 0.4 M NaCl, 0.2% SDS, and 100 ug/ml yeast tRNA), heated at 70 °C for 10 min, then cooled on ice. The nitrocellulose filter blotted with chick GAPDH cDNA was soaked in this poly A^+-RNA solution at 50 °C for 2 h, then washed with 10 mM Tris-HCl, pH 7.6, 0.15 M NaCl, 1 mM EDTA, 0.5% SDS at 65°C. To elute the hybridized mRNA, the filter was transferred to an Eppendorf tube in 300 ul distilled water containing 30 ug yeast tRNA, boiled for 1 h, then frozen quickly in liquid nitrogen. After thawing, the filter was removed and the eluted mRNA was extracted with an equal volume of phenol/chloro-form/isoamyl alcohol (1 : 0.96 : 0.04, vol/vol), then collected by ethanol preci-pitation. *In vitro* translation of the eluted mRNA was performed according to the manufacturer's instructions.

Northern Blot Hybridization--- Total cellular RNA was isolated by the guani-dine thiocyanate method (10). Total RNA 10 ug was separated on a 1% agarose-folmaldehyde gel and transferred to a nylon membrane (GeneScreen Plus, NEN Research Products). Filters were washed with 2x SSC, baked, and hybridized in a solution containing 50% formamide, 5x SSC, 0.6x Denhardt's solution, 1% SDS, and 100 ug of salmon sperm DNA per ml. The denatured ^{32}P-labeled cDNAs for chick GAPDH, human HSP70, mouse β-actin or mouse 28S rRNA were added to the solution for an additional 16 h at 42 °C. Filters were washed in 2x SSC, 0.5% SDS/2x SSC, and then 0.1x SSC. These were dried and exposed to X-ray film.

RESULTS

37-kDa protein is very abundant and consists of several spots on 2-D gels at an isoelectric point of about 8.8 (3). GAPDH had similar characteristics to our 37-kDa protein in terms of molecular size and isoelectric point. We thus compared rabbit GAPDH (Sigma) by peptide mapping using V8 protease, with the 37-kDa proteins purified from mouse 3T3 cells and from chick embryo fibroblasts (Fig. 1). The digestion patterns of these three proteins were not identical but very closely related.

Mouse mRNA for GAPDH was purified by hybrid-selection using the cDNA for chick GAPDH and translated *in vitro* as described in Materials and Methods. It was found that the translated product exhibited the same mobility

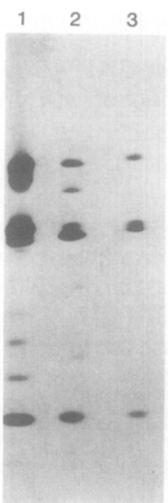

Figure 1. Peptide mapping of mouse and chick 37-kDa proteins and rabbit GAPDH. The 37-kDa protein of BALB/c 3T3 cells (lane 2) and chick embryo fibroblasts (lane 3) were excised from the two-dimensional gels, digested with 0.1 μg/ml of V8 protease, and separated on a SDS-15% polyacrylamide gel. Rabbit GAPDH purchased from Sigma was also digested and separated as described above (lane 1). The peptide fragments were visualized by silver staining methods.

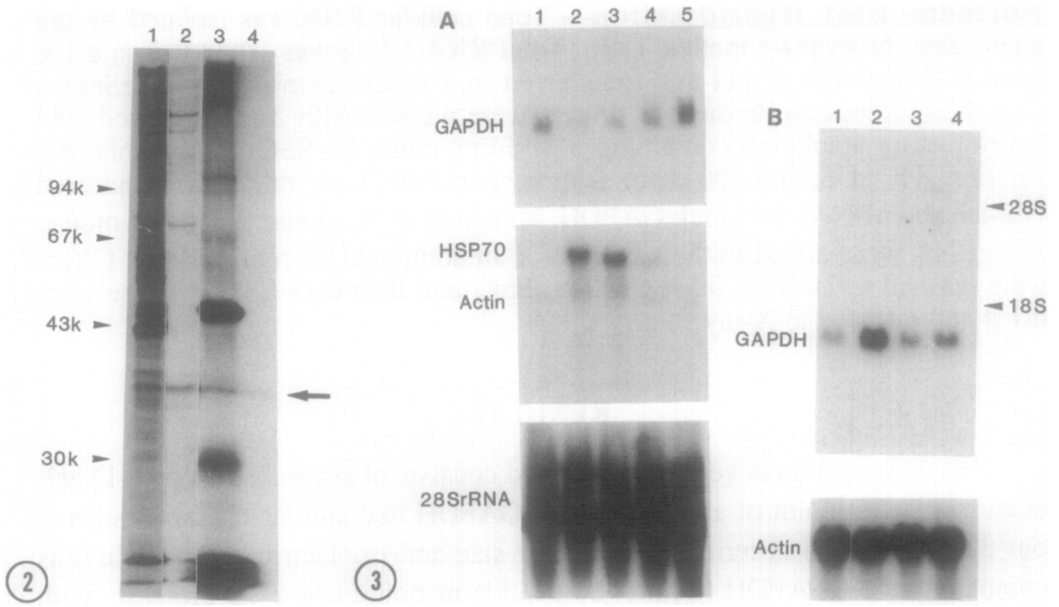

Figure 2. Comparison of the mouse 37-kDa protein with the translated product from the mRNA isolated by hybrid-selection from mouse RNA using a cDNA encoding chick GAPDH. Total cell lysates (lane 1) of BALB/c 3T3 cells labeled with [^{35}S]methionine and its ATP-binding fraction (lane 2) were loaded onto a SDS-10% polyacrylamide gel. Mouse mRNAs for GAPDH was isolated from mRNAs of BALB/c 3T3 cells by hybrid-selection using chick cDNA as described in Materials and Methods, and translated in the presence of [^{35}S]methionine *in vitro*. The total translated product (lane 3) and ATP-binding fraction (lane 4) were electrophoresed. Arrowhead indicates the position of the 37-kDa protein and the *in vitro* translated product.

Figure 3. Induction of mRNA for GAPDH by heat-shock and other reagents. (A) Time course of the induction of mRNA for GAPDH by heat-shock. BALB/c 3T3 cells were heat-shocked at 43 °C for 1.5 h, then allowed to recover for 2 h (lane 3), 4 h (lane 4), and 8 h (lane 5) at 37 °C. Cells were harvested and the total RNAs were prepared by the method described by Chirgwin et al. (10). Total RNAs were also prepared from the control cells without heat shock (lane 1) and from the cells immediately after heat shock (lane 2). Total RNA 10 μg was applied to Northern blot analysis and detected using a cDNA encoding chick GAPDH as a probe. A cDNA encoding for human HSP70 was used to compare the kinetics of the induction of HSP70 with that of GAPDH after heat treatment. Actin cDNA and 28S rRNA cDNA were used as the internal control. (B) BALB/c 3T3 cells (lane 1), the cells treated with 0.3 mM α, α'-dipyridyl for 8 h (lane 2) or 100 ng/ml PMA for 2 h (lane 3), and SV40-transformed cells (lane 4) were collected and analyzed by Northern blotting as in (A).

as the 37-kDa protein and also bound to ATP-agarose as did the 37-kDa protein (Fig.2 and reference 11).

We examined whether or not GAPDH in BALB/c 3T3 cells is in fact induced by heat-shock and several other reagents. We performed Northern blot analysis using cDNA encoding chick GAPDH as a probe, and found that

GAPDH mRNA was induced by heat-shock (Fig. 3A) and drastically by α, α'-dipyridyl, an iron chelating reagent (Fig. 3B). The time course of the induction was clearly different between HSP70 and GAPDH (Fig. 3A). HSP70 mRNA was rapidly induced after heat treatment and quickly disappeared while the induction of GAPDH mRNA was very slow. In BALB/c 3T3 cells transformed by SV40, the mRNA for GAPDH was also increased (Fig. 3B, lane 4). These were consistent with the observations of the 37-kDa protein reported in our previous paper (3). Slight induction of mRNA was also observed in TPA-treated cells, which the 37-kDa protein was also reported to do (Fig. 3B, lane 3, and ref. 3). Thus, we concluded that the 37-kDa protein was identical to GAPDH and that GAPDH was induced by heat shock and by several types of stress in BALB/c 3T3 cells.

DISCUSSION

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a glycolytic enzyme. Two other glycolytic enzymes, enolase (12) and phosphoglycerate kinase (13), are induced by heat shock in yeast. GAPDH is also reported to be heat-inducible in *Xenopus* embryos (14). In human cells, GAPDH mRNA is reportedly induced by treatment with a calcium ionophore as is glucose regulated protein, GRP78 (15). This report provides the first evidence that a mammalian glycolytic enzyme is induced by heat shock.

Induction of three glycolytic enzyme by heat shock draws the conclusion that glycolysis is necessary in cells affected by heat shock. One of the effects of heat shock is the reduction of intracellular ATP by disrupting the normal coupling of electron transport. This causes a shift in energy metabolism from aerobic to glycolytic (14), which may trigger the induction of the glycolytic enzymes to compensate for the depletion of ATP. The slow induction of GAPDH after heat shock may support this possibility. The drastic induction of GAPDH by an iron chelating reagent may be also a result of the oxidative phosphorylation process being affected because α, α'-dipyridyl chelates the ferric ions of the enzymes involved in this pathway.

The expression of GAPDH is reported to be induced by insulin in adipocyte and hepatoma cell lines derived from insulin-sensitive tissues, liver and fat (16). Insulin promotes energy storage and cell growth and also counteracts the effect of catabolic hormones. The effect of insulin is likely to be mediated by altering gene expression, such as that of the GAPDH gene (16). Higher expression of GAPDH in transformed cells may also reflect the faster rate of cell growth than that of normal cells.

The upstream regions of the enolase and phosphoglycerate kinase genes contain imperfect copies of heat-shock element (HSE) (13). Although completely conserved HSE was not detected in the promotor region of the GAPDH

gene (17, 18), it remains to be determined whether a HSE-like element, which can respond to heat-shock, exists in the GAPDH gene of mammalian cells.

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REFERENCES

1. Lindquist, S. and Craig, E.A. (1988) Annu. Rev. Genet. 22, 631-677.
2. Welch, W.J. (1990) In Stress Proteins in Biology and Medicine (R.I. Morimoto, A. Tissieres, and C. Georgopoulos, Eds.) pp. 223-278. Cold Spring Harbor Laboratory Press, New York.
3. Nakai, A., Hirayama, C., Ohtsuka, K., Hirayoshi, K. and Nagata, K. (1990) J. Cell. Physiol. 143, 577-589.
4. Dugaiczyk, A., Haron, J.A., Stone, E.M., Dennison, O.E., Rothblum, K.N. and Schwartz, R.J. (1983) Biochemistry 22, 1605-1613.
5. Wu, B., Hunt, C. and Morimoto, R.I. (1985) Mol. Cell. Biol. 5, 330-341.
6. Tokunaga, K., Taniguchi, H., Yoda, K., Shimizu, M. and Sakiyama, S. (1986) Nucleic Acids Res. 14, 2829.
7. Cleveland, D.W., Fischer, S.G., Kirschner, M.W. and Laemmli, U.K. (1977) J. Biol. Chem. 252, 1102-1106.
8. Morrissey, J.H. (1981) Anal. Biochem. 117, 307-310.
9. Parnes, J.R., Velan, B., Felsenfeld, A., Ramanathan, L., Ferrini, U., Appella, E. and Seidman, J.G. (1981) Proc. Natl. Acad. Sci. USA 78, 2253-2257.
10. Chirgwin, J.M., Pryzybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294-5299.
11. Kamps, M.P., Taylor, S.S. and Sefton, B.M. (1984) Nature 310, 589-591.
12. Iida, H. and Yahara, I. (1985) Nature 315, 688-690.
13. Piper, P.W., Curran, B., Davies, M.W., Lockheart, A. and Reid, G. (1986) Eur. J. Biochem. 161, 525-531.
14. Nickells, R.W. and Browder, L.W. (1988) J. Cell Biol. 107, 1901-1909.
15. Chao, C.C.-K., Yam, W.-C. and Lin-Chao, S. (1990) Biochem. Biophys. Res. Commun. 171, 431-438.
16. Alexander, M.C., Lomanto, M., Nasrin, N. and Ramaika, C. (1988) Proc. Natl. Acad. Sci. USA 85, 5092-5096.
17. Stone, E.M., Rothblum, K.N., Alevy, M.C., Kuo, T.M. and Schwartz, R.Z. (1985) Proc. Natl. Acad. Sci. USA 82, 1628-1632.
18. Ercolani, L., Florence, B., Denaro, M. and Alexander, M. (1988) J. Biol. Chem. 263, 15335-15341.