The Effects of Glucose on Protein Synthesis and Thermosensitivity in Chinese Hamster Ovary Cells*

(Received for publication, June 24, 1983)

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Glucose deprivation induces the major glucose regulated proteins (GRPs) in Chinese hamster ovary cells. When these cells are then returned to a glucose containing environment, GRP synthesis is repressed while concurrently other proteins, identified as heat shock proteins, are induced. The induction of the GRPs is found to mark precisely the onset of a decline in the cell's ability to survive a thermal stress while the expression of heat shock proteins, when glucose is restored, is paralleled by significant increases in survival protection or thermotolerance.

Changes in a cell's environment can affect its ability to proliferate and survive and it is not surprising that cells have inherent mechanisms to adjust themselves to environmental changes. Cellular response to a thermal challenge has been extensively examined and it is well known that a hyperthermic pretreatment can confer a significant protection to a second severe heat challenge (1). The expression of this protection phenomenon has been observed to correlate well with the induction of heat shock proteins resulting from the pretreatment (2-4). The depletion of glucose in a cell's growth medium also imposes a chemical stress to which the cell must accommodate itself and it is well known that glucose deprivation induces the synthesis of two major GRPs of approximately 76 and 97 kDa (5-8). In this report, we examine the effect of GRPs on survival using a thermal challenge as an endpoint. We also examine the response of glucose depleted, GRP-induced cells when returned to a glucose containing environment.

EXPERIMENTAL PROCEDURES

CHO cells obtained from Los Alamos National Laboratory were maintained as monolayer cultures at 37 °C in Ham's F-10 Medium (Gibco) supplemented with 15% newborn calf serum. All experimentation was performed in T-25 flasks (Corning) using asynchronous log phase cells.

To achieve glucose depletion, 15% newborn calf serum was added to F-10 media which was glucose deficient (Gibco) resulting in a broth partially depleted in glucose. Cultures were labeled for 1 h with [35S] methionine (Amersham, 1000 Ci/mmol) prior to and at 4-h intervals following addition of the new medium. Following labeling, cells were washed 2 times with F-10 without serum at 4 °C, resuspended in Hanks' balanced salt solution with 1 mM tosyl-L-arginine methyl ester, 5 mM ethylenediaminetetraacetic acid (Sigma), 1 mM phenylmethylsulfonyl fluoride (Sigma) and sonicated at 4 °C. Protein determinations were made on lysate which was then precipitated with trichloroacetic acid (Fisher Scientific) (10%), neutralized, and dissolved in sodium dodecyl sulfate (Accurate Chemical and Science Corp.) sample buffer. The protein synthesis assay employed discontinuous SDS polyacrylamide gel electrophoresis (12.5%) based on the buffer system of Laemmli (9). Coomasie blue-stained gels were photographed prior to being dried onto filter paper for autoradiography. In all radiograms depicted in this paper, a 24-h exposure of the dried gel to Kodak XAR-5 film was used. Various molecular weights assigned to our proteins were estimated based on comparison with Bio-Rad High and Low Molecular Weight Standards. At each time point, the glucose levels in the medium were monitored using the Sigma glucose colorimetric assay.

At the same 4-h intervals at which protein synthesis was assayed, a 45 °C, 15-min heat shock was delivered to an identical but separate set of cultures by horizontal submersion of flasks into a Haeke PK-2 water bath (+0.1 °C). These cells were then trypsinized and plated for single cell survival as described (2).

In release from glucose depletion experiments, the glucose-depleted media was removed and full media containing glucose and 15% serum was added. Protein synthesis was assayed as just described at 2, 4, 6, 8, and 24 h after release. In this case, the resultant autoradiograms were quantified using a gel scanner (Quick Scan Electrophoresis Dosimeter, Helena Laboratories). The relative percent of a specific protein was determined from the area under the peak (with correction for background density) divided by the total area under the gel scan. Cells survival was also assayed at 0, 2, 4, 6, 8, 12, 15, and 20 h after the glucose shock by applying a 45 °C, 27-min heat challenge. Repli- cation of release experiments by restoring glucose to depleted media or by adding glucose containing media in the absence of serum resulted essentially in the same response.

RESULTS AND DISCUSSION

The pattern of protein synthesis in CHO cells after the addition of partially glucose-depleted media is shown in Fig. 1. The glucose level in the medium decreases with time of incubation and the medium becomes totally depleted after 20 h. After an additional 16 h (at t = 36 h), proteins of 76 and 97 kDa are seen to be strongly induced and resemble the GRPs observed by others. In addition, other induced proteins are evident at 135 and 14.4 kDa. This altered protein synthesis pattern continues to the termination of the experiment at t = 56 h. In addition, these deprivation studies resulted in no significant reduction in total cell protein synthesis (cpm/mg of protein). When cells are challenged at the same times by a 45 °C, 15-min heat treatment (Fig. 2), little variation in colony forming ability is observed initially, even at times following 20 h when glucose had been depleted from the media. However, the onset of a significant increase in the sensitivity to a heat dose is observed to occur between 32 and 36 h, at the precise time at which the major GRPs appear. During the subsequent period of GRP synthesis, a continual reduction in colony forming ability is observed. During this entire period of glucose depletion, no change occurred in the ability of cells to form colonies in the absence of heat stress.

When glucose deficient medium is then replaced (at t = 56 h) by glucose containing medium, the resultant effect on protein synthesis is shown in Fig. 3. In this instance, a gradual

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‡The abbreviations used are: GRPs, glucose regulated proteins; CHO, Chinese hamster ovary fibroblasts; HSPs, heat shock proteins; SDS, sodium dodecyl sulfate.
Glucose-regulated and Heat Shock Proteins

This glucose shock results in no significant alteration in total cell protein synthesis. The induction of HSP 89 and HSP 68 together with the decline in synthesis of GRP 97 and GRP 76 from the induced state are presented in quantitative form in Fig. 4. When cells are challenged (45 °C, 27 min) at various times following exposure to glucose, an initial thermal sensitization relative to the sensitivity of control cultures is seen (0 h, Fig. 5). At subsequent times, a recovery to control levels of sensitivity is evident followed by the additional expression of significant levels of protection. The release from a GRP-induced state, therefore, results in the induction of at least some heat shock proteins and in the associated protection phenomenon of thermotolerance.

This apparent inverse relationship between GRPs and heat shock proteins, thermosensitization and protection, may also result from the introduction of other environmental states. We have also observed by two-dimensional analysis that a 16-h anaerobic exposure in a Brewer jar induces the identical 97- and 76-kDa GRPs (10). It is already recognized that a significant thermosensitization in CHO cells is generated by an almost identical anaerobic exposure (11) and it is also established that release from anoxia induces heat shock proteins in a number of organisms (12).

It has recently been reported that glucocorticoids can elicit a heat shock-like response in glucose-deprived cells (13). The possible stimulation of gluconeogenesis in this instance could result in an increase in intracellular glucose levels and may, therefore, parallel some of the observations reported in this study. These authors further point out that glucose depletion and GRP induction may result from routine culture schedules. Concomitant changes in cell sensitivity to heat and other stimuli, as indicated here, may also result. In addition, since transformed cells can utilize glucose more rapidly and express high levels of GRPs (5, 7) (previously considered as transformation sensitive proteins), the results reported here may explain the enhanced thermosensitivity often expressed by transformed cells relative to their untransformed counterparts (14, 15). The function of glucose regulated proteins is unknown, but it is possible that they also protect the cell against an alternate stressor which differs from heat or other heat shock inducing stresses.

**Fig. 1. Glucose depletion: protein synthesis.** Glucose was totally depleted at 20 h and the major GRPs are seen at 36 h. Hours of incubation at 37 °C following addition of partially depleted media is indicated (top) with the Coomassie blue-stained gel represented (left) together with the autoradiogram (right). At each time point, the glucose levels in the media were monitored using the Sigma glucose colorimetric enzymatic assay. Right margin indicates major GRPs at 97 and 76-kDa. Two other induced proteins are indicated. Left margin indicates molecular weight standards (top to bottom): β-Galactosidase (116,000), phosphorylase b (92,500), bovine serum albumin (66,200), and ovalalbumin (43,000). Equal masses of protein were loaded in each lane using the Bio-Rad protein determination assay.

**Fig. 2. Glucose depletion: cell survival.** Total glucose depletion is observed at 20 h (A) and the major GRPs appear between 32 and 36 h (B), concurrent with a significant fall off in survival. Error bars: ± standard error of mean.

Reduction in synthesis of GRPs is evident. At the same time, an accelerated synthesis of a 68-kDa protein above control levels is observed. This induced protein co-migrates on a two-dimensional gel (isoelectric focusing/SDS-polyacrylamide gel electrophoresis) with the major CHO cell heat shock protein which occurs at this molecular weight (data not shown). Inductions of 89- and 110-kDa proteins, which also resemble the heat shock proteins of these molecular weights, also occur.

*J. Sciandra, C. Hughes, and J. Subjeck, unpublished data.*
**FIG. 3. Release from glucose depletion: protein synthesis.** The induction of HSP 68 is specifically evident along with more modest inductions of HSP 89 and 110. In this case, protein synthesis was examined at the hours indicated (top) after addition of full media. (C, control). The Coomassie blue-stained gel is represented (left) together with its autoradiogram (right). Right margin, the position of migration of HSPs and GRPs discussed in text. Left margin, molecular weight standards as in Fig. 1. Equal masses of protein were loaded.

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**FIG. 4. Release from glucose depletion: quantitation of protein synthesis.** Each point is the amount of labeled [35S]methionine incorporated into protein in 1 h. Glucose containing media added at time zero. Shown are GRP 97 (○), GRP 76 (●), HSP 89 (×), and HSP 68 (□). In control cultures, the per cent of protein synthesized per h is: GRP 97, 1.3%; GRP 76, 0.5%; HSP 89, 2.0%; HSP 68, 1.2%; actin, 6.1%. Actin synthesis varied by less than ±10% during this release period.

**FIG. 5. Release from glucose depletion: cell survival.** Cells are initially sensitized to a heat dose relative to control cultures (0 h) but rapidly recover to control levels of resistance (2 h) and then acquire a significant level of protection to a heat challenge at later times. Survival of control cells is indicated (▲). Error bars: ± standard error of mean.
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J. Biol. Chem. 1983, 258:12091-12093.

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