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Biochemical Characterization of Glucose-Regulated Proteins, Grp94 and Grp78/BiP

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Glucose-regulated proteins (grp's), grp94 and grp78/BiP, are a group of stress proteins which are highly synthesized in cells exposed to a variety of stressful agents including tunicamycin and Ca^{2+} ionophore. Grp78/BiP is known to function as a molecular chaperone which regulates the folding and assembly of secretory or membrane proteins, but the biological function of grp94 remains to be elucidated. In this study, we have examined the intracellular distribution of grp's and the function of grp94. Grp's are resident in the endoplasmic reticulum (ER) and a specific sequence (Lys-Asp-Glu-Leu) at their C-terminus is known to be responsible for their retention within the ER. However, it has been unclear whether upon disturbance of cellular Ca^{2+} homeostasis by the Ca^{2+} ionophore, grp94 is retained within the ER or secreted into the medium. In this study, we showed that in the presence of Ca^{2+} ionophore, grp94 and grp78/BiP are present in the cells, mainly within the ER. We have also investigated whether grp94 might function as a molecular chaperone. Here we showed that in the immunoglobulin (Ig)-secreting hybridoma cells, grp94 transiently interacts with fully glycosylated Ig heavy chain, suggesting that grp94 may be involved in facilitating the folding and assembly of Ig heavy chains.

KEY WORDS: A23187, Grp94, Grp78/BiP, Endoplasmic Reticulum, Immunoglobulin Heavy Chain

Glucose-regulated proteins (grp's) were originally discovered as a set of proteins induced specifically by glucose starvation (Poussegur *et al.*, 1977) and subsequently found to be also induced in the cells subjected to a variety of stressful conditions including tunicamycin and agents which perturb Ca^{2+} homeostasis (Welch *et al.*, 1983; Lindquist, 1986; Kang and Welch, 1991; Lee, 1992). Most of grp's, however, are also expressed in considerable amounts in the normal unstressed cells, suggesting the role of grp's in the very basic and essential function of the normal cells (Welch *et al.*, 1991).

Grp protein family includes grp94 and grp78/BiP which are resident endoplasmic reticulum (ER) proteins (Welch *et al.*, 1991). Grp's are structurally and functionally related to the heat shock proteins (hsp's), whose levels increase in response to treatment such as heat shock and exposure to heavy metals. Grp78/BiP exhibits considerable sequence homology to cytosolic hsp72 and hsp73 (Munro and Pelham, 1986) and like hsp72/73, it functions as a molecular chaperone which regulates *in vivo* folding and assembly of many membrane or secretory proteins in the ER (Bole *et al.*, 1986; Blount and Merlie,

1991; Goething and Sambrook, 1992). Similarly, grp94, an endoglycosidase H-sensitive glycoprotein, is related to cytosolic hsp90 (Sargan *et al.*, 1986; Mazzarella and Green, 1987) and is also suspected to act as a molecular chaperone. Recently, it has been shown that grp94 associates with unassembled major histocompatibility complex class II polypeptides, mutant herpes glycoproteins and immunoglobulin (Ig) heavy chains (Navarro *et al.*, 1991; Lee, 1992; Melnick *et al.*, 1994). In addition, it was reported that grp94 interacts, in a Ca^{2+}-dependent manner, with grp78/BiP (Kang and Kim, 1992).

Grp's possess a specific sequence (Lys-Asp-Glu-Leu; KDEL) at their C-terminus which is responsible for the ER retention of grp's (Munro and Pelham, 1986; Mazzarella and Green, 1987). The retention of grp's involves a KDEL receptor which serves to induce retrieval transport to ER of grp's which have escaped from the ER. Recently, Vaux *et al.* (1990) have reported the detection of KDEL receptors in the intermediate compartment which is located between the ER and the *cis*-Golgi.

With respect to the intracellular retention of grp's, Booth and Koch (1989) reported that a number of resident ER proteins including grp's were secreted into the medium when murine fibroblasts were long term treated with the Ca^{2+} ionophore (A23187) and that the secreted form of grp94 was endoglycosidase H-resistant, thereby indicative of its further modification by additional carbohydrates. However, it was also demonstrated that treatment of cells with A23187 did not cause either the secretion of grp94 into the medium or its modification to an endoglycosidase H-resistant form (Lodish and Kong, 1990; Kang and Welch, 1991). Consequently whether upon treatment of cells with the Ca^{2+} ionophore, grp's pass through the normal secretory pathway or are retained within the ER requires additional studies.

In an attempt to resolve this issue, we have examined the secretion of grp's by the Ca^{2+} ionophore by endoglycosidase H-sensitivity analysis, immunofluorescence microscopy, and subcellular fractionation experiments. Here we present evidences supporting that in the presence of Ca^{2+} ionophore, grp94 is neither secreted into the medium nor modified to an endoglycosidase H-resistant form.

In this study, we have also examined whether grp94 might possess the molecular chaperone activity. Here, we show that in the Ig-secreting hybridoma cells, grp94 is complexed with grp78/BiP and transiently interacts with Ig heavy chain, thereby indicative of a possible role of grp94 as a molecular chaperone.

Materials and Methods

Cell culture

HeLa cells and Ig-secreting hybridoma cells were cultured in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% calf serum or RPMI 1640 supplemented with 10% fetal calf serum, respectively. Skeletal muscle cell culture was done using breast myoblasts of 12 day-old chick embryos according to the method of Ha *et al.* (1983). The muscle cells were cultured in Eagle's essential medium (MEM) plus 10% horse serum, 10% chick embryo extract and 1% antibiotic/antimycotic solution (811 medium) for 24 h after plating and then cultured in the same medium but containing 2% chick embryo extract (8102 medium).

Metabolic labeling and pulse chase experiment

For [^{35}S]methionine labeling, cultured cells were washed in methionine-free DMEM once and labeled in methionine-free DMEM plus [^{35}S] methionine supplemented with 2% dialyzed calf serum. For the pusle-chase experiment, cultured cells were rinsed in methionine-free DMEM and pulse-labeled with [^{35}S]methionine for 10 min. After the labeling, one of the plates was immediately harvested and the others were chased in the complete media for various times. The labeled cells were washed in cold phosphate-buffered saline (PBS) three times and harvested in appropriate buffers.

One dimensional and two dimensional gel eletrophoresis

Labeled cells were lysed in sodium dodecyl sulfate (SDS)-Laemmli sample buffer (40 mM Tris-

HCl, pH 6.8, 1% SDS, 50mM dithiothreitol, 7.5% glycerol, 0.003% bromophenol blue) and the cellular proteins were analyzed either by one dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE) or by two dimensional gel electrophoresis employing pH 5-7 (80%), 3-10 (20%) ampholines in the isoelectric focusing dimension, and 12.5% SDS-polyacrylamide gel in the second dimension (Laemmli, 1970; Garrels, 1979), followed by fluorography.

Western blotting

Polyclonal anti-grp94 were prepared in the rabbit against purified grp94 as described in Kang and Welch (1991) and polyclonal anti-grp75 was provided by Dr. Welch (Univ. of California, S.F.).

Western blotting was carried out according to the procedure of Towbin *et al.* (1979). The proteins separated by SDS-PAGE was transferred to nitrocellulose paper electrophoretically and then the paper was treated with appropriate antibody and then alkaline phosphatase-conjugated secondary antibody. After extensive washing, color development of the blots was performed.

Immunoprecipitation

For the immunoprecipitation, labeled cells were solubilized in RIPA(+) buffer (1% Triton X-100, 1% deoxycholate, 0.1% SDS in PBS). For the immunoprecipitation for the detection of grp94 complexed with other proteins, labeled cells were lysed in RIPA(+) containing apyrase which removes endogeneous ATP. Following preabsorption in Sepharose 4B at 4°C for 30 min, the cell lysate was incubated with appropriate antibody at 4°C for 2 h. The immune complexes were captured by protein A-agarose and the immunoprecipitate was washed with RIPA(+) 5 times and the proteins were then released by boiling in Laemmli sample buffer and analyzed by SDS-PAGE and fluorography.

Endoglycosidase H digestion

Labeled cells were lysed in RIPA(+) buffer and the cell lysates were then immunoprecipitated with anti-grp94. The immunoprecipitated grp94 was released from the protein A-Sepharose antibody complexes by boiling in Laemmli sample buffer at 100°C for 5 min. An aliquot of the released materials was adjusted to 0.15% SDS, 20 mM sodium citrate, pH 5.5, 10mM EDTA, and 75 μM 2-mercaptoethanol. Endoglycosidase H was added (final 10 units/ml) and the sample incubated at 37°C for 16 h. Following the incubation, Laemmli sample buffer was added and boiled at 100°C and the proteins analyzed by SDS-PAGE and fluorography.

Subcellular fractionation by sucrose-gradient centrifugation

Labeled cells were washed with cold PBS, and incubated in 10 volumes of cold hypotonic buffer (5 mM Hepes, pH 7.4) and lysed with 10 gentle strokes in a tight-fitting dounce homogenizer and the solution was immediately adjusted to 0.25 M sucrose, 1 mM $MgCl_2$ and nuclei and cellular debris removed by centrifugation at 800 $\times$g for 10 min. The postnuclear supernate was then layered on top of a discontinuous sucrose gradient containing 1 ml/2.0 M, 3.4 ml/1.3 M, 3.4 ml/1.0 M, and 2.75 ml/0.6 M sucrose in 5 mM Hepes (pH 7.4) in a Beckman 14 $\times$ 89 mm Ultra-Clear tube. After 2 h centrifugation at 40,000 rpm in a Beckman SW41 rotor, 19 fractions containing 10 drops each were collected from the bottom of the tubes. Membranes enriched for rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), and Golgi apparatus were recovered at the three interfaces of the sucrose layers after centrifugation (Bole *et al.*, 1986). Fractions enriched in RER, SER, Golgi or cytosol were either immunoprecipitated with appropriate antibody or precipitated by ATP-agarose beads. The precipitated proteins were analyzed by SDS-PAGE and fluorography.

ATP-agarose binding assay

ATP-agarose was washed and stored at 4°C as a 1:1 solution in buffer B (20 mM Tris-HCl, pH 7.4, 20 mM NaCl, 0.1 mM (ethylenedinitrilo)tetraacetic acid, 2 mM dithiothreitol). Labeled cell lysates in RIPA(+) were mixed with 200 μl ATP-agarose, and the samples were rocked at 4°C for 6-8 h. The ATP-agarose beads were pelleted by centrifugation and the unbound material was

removed and the ATP-agarose beads were washed three times with buffer B. And the materials bound to ATP-agarose beads were eluted by boiling in SDS-Laemmli sample buffer at 100°C. The proteins were analyzed by SDS-PAGE and fluorography.

Immunofluorescence microscopy

Chick embryonic muscle cells were cultured on the cover slip in the presence or absence of A23187. The cells were then fixed with 4% paraformaldehyde and permeabilized with 0.2% Triton X-100. After rinsing, the cells were incubated with anti-grp94 polyclonal antibody and FITC-conjugated anti-rabbit IgG and the cells were subjected to immunofluorescence microscopy analysis.

Results and Discussion

Induction of grp94, grp78/BiP and grp75 by treatment of Ca^{2+} ionophore

As others have shown, and as illustrated in Fig. 1, when HeLa cells were exposed to the Ca^{2+} ionophore (A23187) perturbing intracellular Ca^{2+} homeostasis, the synthesis of grp94 and grp78/BiP was markedly induced, whereas the synthesis of actin and other stress proteins such as hsp90, hsp72/73, and hsp60 was not changed. By Western blot analysis using polyclonal anti-grp94 antibody (Kang and Welch, 1991; Kang and Kim, 1992) and ATP-agarose binding assay, it was confirmed that the synthesis of grp94 and grp78/BiP was highly enhanced by treatment of the Ca^{2+} ionophore (Fig. 2). ATP-agarose binding assay was carried out to detect hsp70 family proteins (hsp72/73 in the cytosol, grp78/BiP in the ER, grp75 in the mitochondria). ATP-agarose binding assay revealed that in addition to grp78/BiP, the synthesis of grp75 which was previously reported to be a mitochondrial grp and equivalent to *E. coli* dna K (Mizzen *et al.*, 1989; Welch *et al.*, 1991) was also induced (Fig. 2B). However, the level of cytosolic hsp73, constitutively expressed in normal cells, is not significantly changed by A23187 treatment. Thus, among hsp70 and hsp90 family proteins, only ER and mitochondrial stress proteins (grp94, grp78/BiP and grp75) appear to be induced by the Ca^{2+} ionophore. These results indicated that the gene expression of grp's might be closely coupled to the level of two intracellular Ca^{2+} stores, the ER and mitochondria.

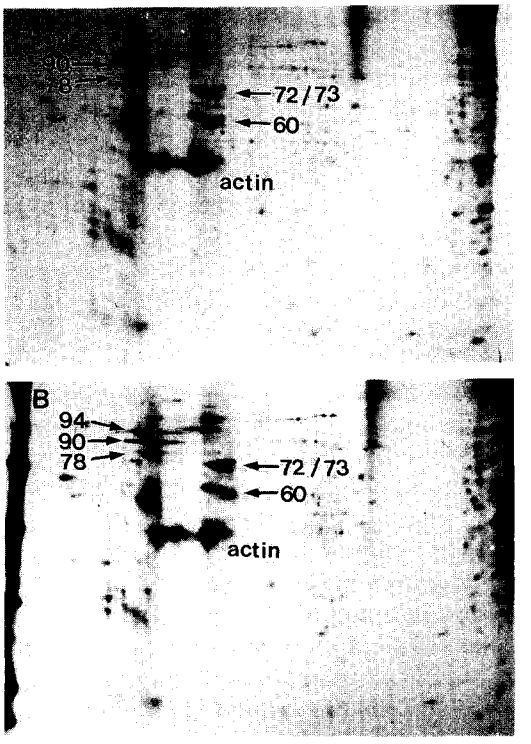

Fig. 1. Induction of grp94 and grp78/BiP by treatment of Ca^{2+} ionophore. HeLa cells were incubated in the absence (panel A) or presence (panel B) of 7 μM A23187 for 6 h and then labeled with [^{35}S]methionine in the absence or presence of A23187 for 4 h. The labeled proteins were analyzed by two dimensional polyacrylamide gel electrophoresis and fluorogrpahy. Grp94, hsp90, grp78/BiP, hsp72/73, hsp60 indicated by arrows.

Intracellular retention of grp's in the presence of Ca^{2+} ionophore

Grp94 and grp78/BiP are resident in the ER and their C-terminal tetrapeptide sequence, KDEL, is known to be responsible for the ER retention of grp's (Munro and Pelham, 1986; Mazzarella and Green, 1987). Furthermore, grp94 is an endoglycosidase H-sensitive glycoprotein

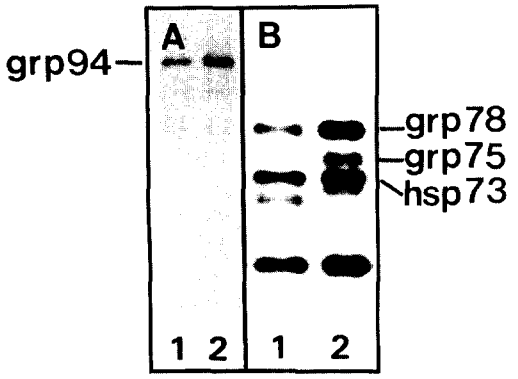

Fig. 2. Western blotting and ATP-agarose binding assay for the induction of grp's by A23187. HeLa cells were exposed to normal growth medium (lane 1) or 7 μM A23187 (lane 2) for 12 h and then labeled with [^{35}S] methionine in the same conditions for 6 h. Equal amounts of the labeled proteins were analyzed either by Western blotting using anti-grp94 (panel A) or by ATP-agarose binding assay (panel B).

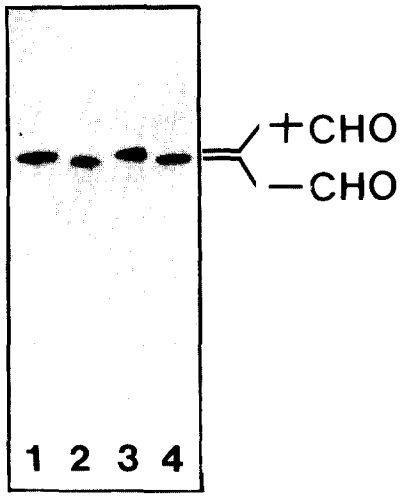

Fig. 3. Endoglycosidase H sensitivity of grp94. HeLa cells were labeled in [^{35}S]methionine for 4 h, the label removed and the cells then incubated for 4 h either in the absence (lanes 1-2) or presence (lanes 3-4) of 7 μM A23187. The labeled cells were lysed in RIPA(+) and the cell lysates then immunoprecipitated with anti-grp94 and the resulting immunoprecipitates were incubated in the absence (lanes 1, 3) or presence (lanes 2, 4) of endoglycosidase H. The proteins were analyzed by SDS-PAGE and fluorography. +CHO, glycosylated grp94; -CHO, deglycosylated grp94.

characteristic of resident ER glycoproteins (Welch *et al.*, 1991; Lee 1992). However, Booth and Koch (1989) have shown that prolonged exposure of mouse 3T3 cells to the Ca^{2+} ionophore resulted in the secretion of grp94 into the medium and its modification to an endoglycosidase H-resistant form. On the contrary, others have demonstrated that the Ca^{2+} ionophore did not cause changes in either the endoglycosidase H sensitivity or the ER retention of grp94 (Lodish and Kong, 1990; Kang and Welch, 1991). Consequently, the question of whether grp's are present in the ER or secreted into the medium upon treatment of A23187 remains controversial.

In order to examine whether grp94 might exit the ER and be modified to an endoglycosidase H-resistant form as a result of perturbation in the intracellular [Ca^{2+}], HeLa cells were labeled in [^{35}S]methionine for 4 h, unlabeled [^{35}S] methionine removed and the cells then incubated for 4 h either in the presence or absence of 7 μM A23187. The labeled cells were lysed in RIPA(+) buffer and the cell lysates were then immunoprecipitated with anti-grp94. The resulting immunoprecipitates were incubated either in the presence or absence of endoglycosidase H. As shown in Fig. 3, grp94 either from normal cells or from A23187-treated cells was all sensitive to endoglycosidase H digestion. Furthermore, when media were analyzed for the grp94 secretion by

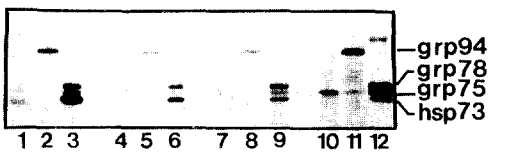

Fig. 4. Subcellular fractionation by sucrose gradient centrifugation. HeLa cells were labeled in [^{35}S] methionine for 6 h at 37 oC, the label removed and the cells then incubated for 4 h in the presence of 7 μM Ca^{2+} ionophore, A23187. Subcellular fractionation was carried out as described in Materials and Methods. Fractions enriched in cytosol (lanes 1-3), Golgi apparatus (lanes 4-6), SER (lanes 7-9), and RER (lanes 10-12) were obtained and then either immunoprecipitated with anti-grp75 (lanes 1, 4, 7, 10) or anti-grp94 (lanes 2, 5, 8, 11) or precipitated with ATP-agarose beads (lanes 3, 6, 9, 12). The proteins were analyzed by SDS-PAGE and fluorography.

treatment of A23187, no obvious secretion of grp94 was observed (data not shown). Thus, in the presence of A23187, grp94 is not either secreted into medium or modified to an endoglycosidase H-resistant form.

Further experiments to examine whether the Ca^{2+} ionophore might cause changes in the ER retention of grp94 were carried out. Two approaches were adopted; subcellular fractionation experiment and immunofluorescence microscopy analysis. For subcellular fractionation experiment, HeLa cells were labeled in [^{35}S] methionine for 6 h, the label removed and the cells then further incubated for 4 h in the presence of 7 μM A23187. The cells were lysed in a dounce homogenizer and then nuclei and cellular debris removed by centrifugation. The postnuclear supernatant was then layered on top of a discontinuous sucrose gradient. After 2 h centrifugation at 40,000 rpm, fractions enriched

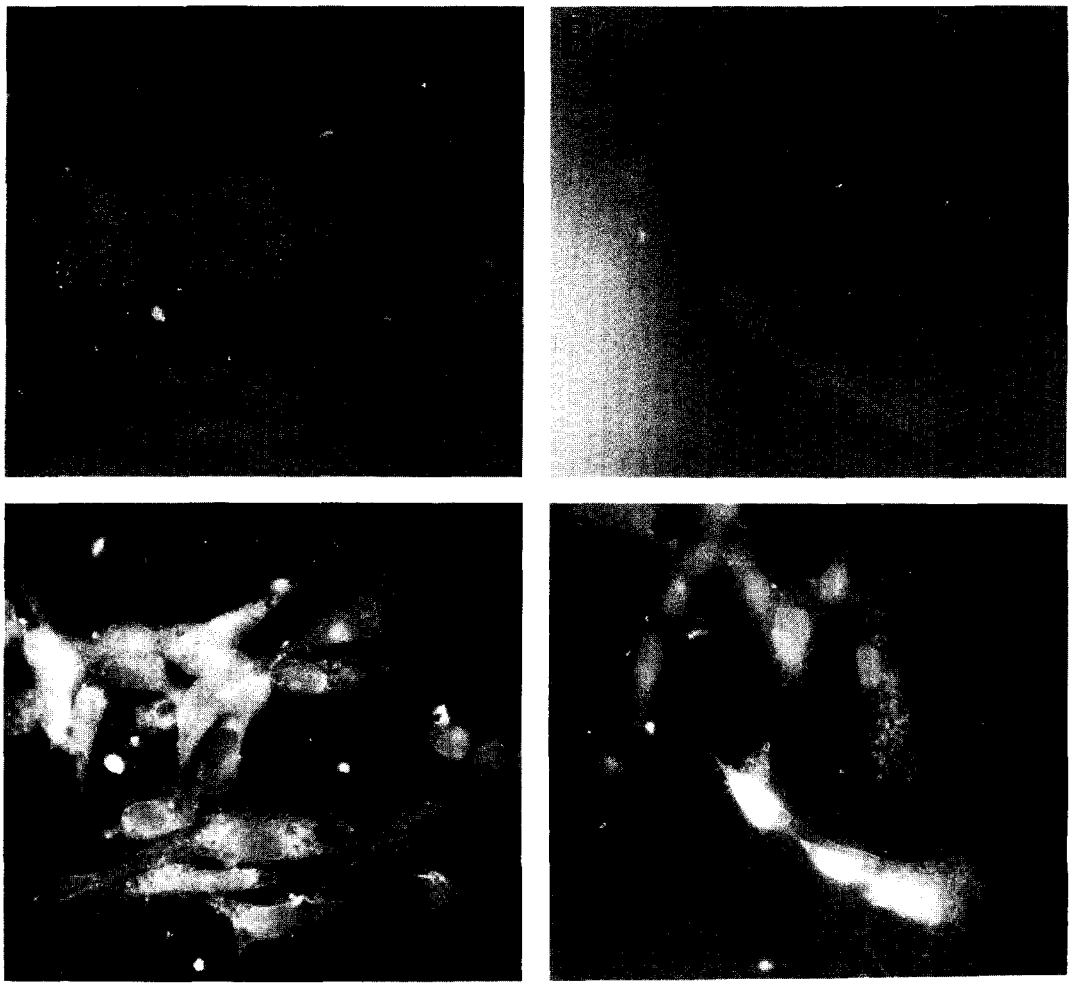

Fig. 5. Intracellular distribution of grp94. Chick embryonic muscle cells were cultured on the cover slip in the absence (panels A and C) or presence (panels B and D) of 7 μM A23187 for 12 h and then permeabilized with Triton X-100 and anti-grp94 polyclonal antibodies and FITC-conjugated anti-rabbit IgG were treated to cells and the the cells were subjected to immunofluorescence microscopy analysis (panels C and D). Panel A and B, phase contrast microscopic photographs of cultured muscle cells. $\times$400.

for rough ER (RER), smooth ER (SER), Golgi, and cytosol were obtained (Bole *et al.*, 1986) and either immunoprecipitated with anti-grp94 or anti-grp75 or precipitated by ATP-agarose beads (Fig. 4). As shown in Fig. 4, most of both grp94 and grp78/BiP were found in RER fractions, although small amounts of them were recovered in the cytosol and other membrane fractions. Grp75, a mitochondrial grp, is also found with grp94 and grp78/BiP in RER fractions, indicating that the mitochondria was cosedimented with RER. However, hsp73 was present mostly in the cytosol. These results indicated that despite disturbance in the intracellular [Ca^{2+}] by treatment of Ca^{2+} ionophore, grp94 and grp78/BiP were present within the cells, mostly in the RER.

In order to further examine the subcellular localization of grp's in the A23187-treated cells, immunofluorescence miscroscopy analysis was carried out. For the immunofluorescence microscopy experiment, chick embryonic myoblasts were used since they grow attached to a culture dish and show a large cell morphology. Chick embryonic myoblasts were cultured on cover slips in the presence or absence of 7 μM A23187 for 12 h and then fixed with paraformaldehyde and permeabilized with Triton X-100. The cells were incubated with anti-grp94 polyclonal antibody and FITC-conjugated anti-rabbit IgG and the cells were subjected to immunofluorescence microscopy analysis. As shown in Fig. 5, in the normal cells, grp94 was distributed in a fine punctate/reticular pattern charateristic of ER staining. Exposure of cells to the Ca^{2+} ionophore did not cause a significant change in the distribution of grp94. Taken together these results, we conclude that grp94 and grp78/BiP are present in the ER and are not secreted into the media in the cells of which intracellular Ca^{2+} homeostasis was disturbed as well as in the normal cells.

Interaction between grp94, grp78/BiP and immunoglobulin heavy chain

Previously we have demonstrated that grp94 specifically associates with grp78/BiP and other proteins (Kang and Kim, 1992). In order to examine whether grp94 interacts with secretory proteins, Ig-synthesizing hybridoma cells were incubated in the absence or presence of 7 μM A23187 or 0.25% 2-mercaptoethanol for 4 h and labeled in the [^{35}S]methionine for 2 h. The labeled cells were lysed in the RIPA(+) plus apyrase and the resulting cell lysates were precipitated with either protein A-agarose, anti-grp94-bound protein A-agarose, or ATP-agarose beads. Anti-grp94-bound protein A-agarose was prepared by incubation of protein A beads with an excess of grp94 to prevent protein A-binding of Ig. As shown in Fig. 6, glycosylated (slowly migrating) and unglycosylated (fastly migrating) Ig heavy chains were precipitated with protein A-agarose

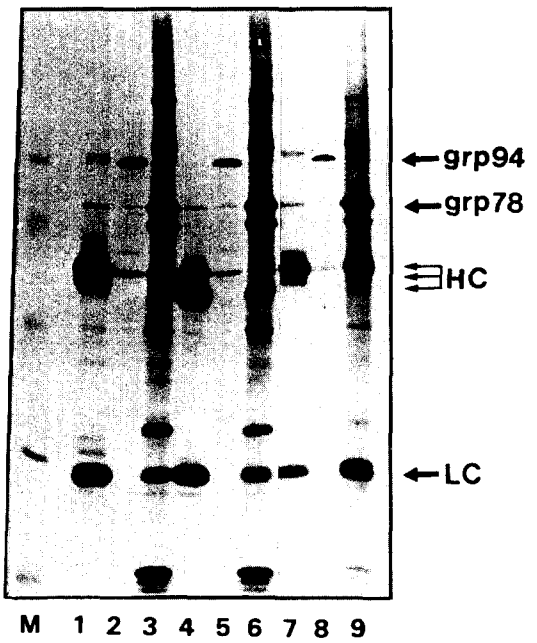

Fig. 6. Association of grp94, grp78/BiP and immunoglobulin heavy chain. Ig-synthesizing hybridoma cells were incubated in the absence (lanes 1-3) or presence of 7 μM A23187 (lanes 4-6) or 0.25% 2-mercaptoethanol (lanes 7-9) for 4 h and labeled in the [^{35}S]methionine in the absence or presence of each of the stress agents for 2 h. The labeled cells were lysed in the RIPA(+) containing apyrase and the resulting cell lysates were precipitated either with protein A-agarose (lanes 1, 4, and 7) or anti-grp94-bound protein A-agarose (lanes 2, 5, and 8), or with ATP-agarose beads (lanes 3, 6, and 9). The proteins were analyzed by SDS-PAGE and fluorography. HC, immunoglobulin heavy chain; LC, immunoglobulin light chain.

and grp78/BiP and Ig light chain were specifically coprecipitated with Ig heavy chain. In the immunoprecipitation with anti-grp94 antibody, proteins which comigrate with grp78/BiP and Ig heavy chain were specifically coprecipitated with grp94. Interestingly, only fully glycosylated, but not partially glycosylated and unglycosylated, Ig heavy chains were coprecipitaed with grp94. In the immunoprecipitation with protein A, grp94 was not coprecipitated with Ig and grp78/BiP. Therefore, to confirm that grp94 interacts with Ig, ATP-agarose binding assay was carried out. ATP-agarose binding assay showed that grp94 and IgG heavy and light chains were coprecipitated with grp78/BiP. Similar patterns were observed in the precipitation experiments using A23187- or 2-mercaptoethanol-treated cells. Thus, grp94 appears to interact with Ig heavy chain as well as grp78/BiP.

Grp78/BiP is known to interacts transiently with Ig heavy chain (Bole *et al.*, 1986). Therefore, to examine whether grp94 interacts transiently with Ig heavy chain during their synthesis within the ER, pulse-chase experiment was carried out. Hybridoma cells were pulse labeled with [^{35}S] methionine for 10 min and chased at various times. The cell lysates were immunoprecipitated with anti-grp94. As shown in Fig. 7, grp94 interacts with glycosylated Ig heavy chain and the association was maximal during chase period from 20 to 40 min. Thus, grp94 appears to interact transiently with Ig heavy chain.

Since grp94 exhibits sequence homology to cytosolic hsp90 which acts as a molecular chaperone (Mazzarella and Green, 1987; Picard *et al.*, 1990; Sanchez *et al.*, 1990), it has been suggested that grp94 may act as molecular chaperone. Recently, grp94 was shown to be associated with some secretory or membrane proteins including Ig heavy chains (Navarro *et al.*, 1991; Lee, 1992). Melnick *et al.* (1994) have shown that grp78/BiP binds Ig heavy and light chains soon after their translocation into the ER and dissociates within few minutes, whereas grp94 associates with Ig heavy chains after grp78/BiP and dissociates with a half-time of 50 min. Based on these results, we propose that in the ER, grp94 participates in the folding and assembly of Ig heavy chains and that two resident ER stress proteins, grp78/BiP and grp94, act in tandom in

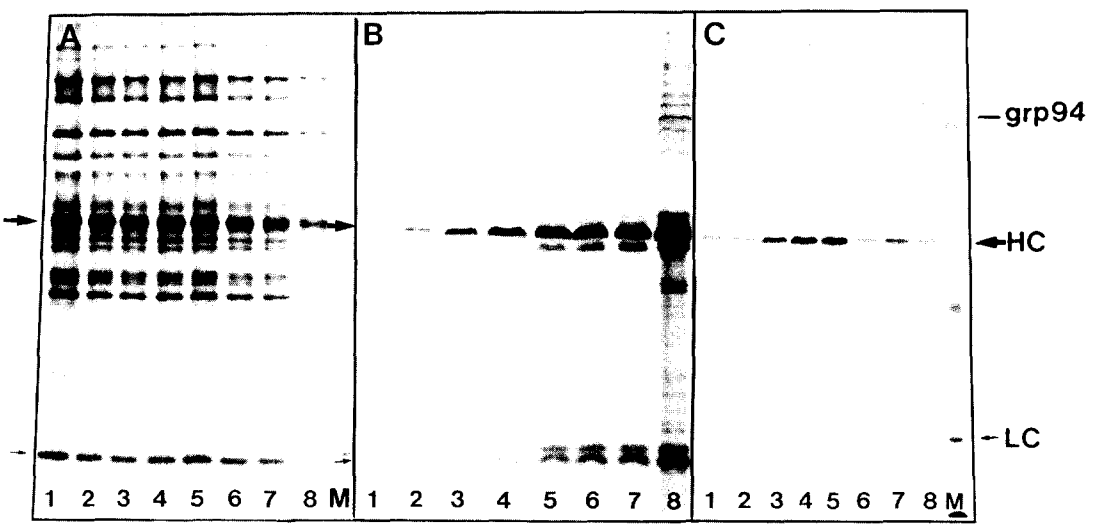

Fig. 7. Transient interaction between grp94 and immunoglobulin heavy chain. Hybridoma cells were pulse-labeled with [^{35}S]methionine for 10 min and chased for 0, 10, 20, 30, 40, 60, 120, and 180 min (lanes 1-8 respectively). The cell lysates were immunoprecipitated with anti-grp94 and the proteins analyzed by SDS-PAGE and fluorography (panel C). Panel A, the cell lysates; panel B, the media. HC, immunoglobulin heavy chain; LC, immunoglobulin light chain. Shown in lane M is molecular weight markers (in descending order are phosphorylase b (97.4 KDa), bovine serum albumin (68 KDa), ovalbumin (43 KDa)).

facilitating folding and assembly of Ig. However, it remains to be eucidated how resident ER chaperones, grp78/BiP and grp94, interact with their substrates and whether they need a cofactor (s) in chaperoning substrate polypeptides.

Acknowledgements

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References

Blount, P. and J.P. Merlie, 1991. BiP associates with newly synthesized subunits of the mouse muscle nicotinic receptor. *J. Cell Biol.* **113:** 1125-1132.

Bole, D.G., L.M. Hendershot and J.F. Kearney, 1986. Posttranslational association of immunoglobulin heavy chain binding protein with nascent heavy chains in nonsecreting and secreting hybridomas. *J. Cell Biol.* **102:** 1558-1566.

Booth C and G.L.E. Koch, 1990. Perturbation of cellular calcium induces secretion of luminal ER proteins. *Cell* **59:** 729-737.

Ha D.B., B.J. Yoo, J.K. Sonn, H.S. Kang, and Y.S. Lee, 1983. Synthesis of muscle specific proteins during the differentiation of chick embryonic muscle cells in culture. *Korean J. Zool.* **26:** 1-17.

Garrels, J.I., 1979. Two-dimensional gel electrophoresis computer analysis of proteins synthesized by clonal cell lines. *J. Biol. Chem.* **254:** 7961-7977.

Goething, M.J., K. McCammon, and J. Sambrook, 1986. Expression of wild type and mutant forms of influenza hemagglutinin: the role of folding in intracellular transport. *Cell* **46:** 939-950.

Kang, H.S. and W.J. Welch, 1991. Characterization and purification of the 94-kDa glucose-regulated protein. *J. Biol. Chem.* **266:** 5643-5649.

Kang, H.S. and H.D. Kim, 1992. Association of 94kDa glucose-regulted proteins and immunoglobulin heavy chain binding protein (BiP). *Kor. Z. Zool.*

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature* **227:** 680-685.

Lee, A.S., 1992. Mammalian stress response: induction of the glucose-regulated protein family. *Curr. Opinion in Cell Biol.* **4:** 267-273.

Lindquist, S., 1986. The heat shock response. *Ann. Rev. Biochem.* **55:** 1151-1191.

Lodish, H.F. and N. Kong, 1990. Perturbation of cellular calcium blocks exit of secretory proteins from the endoplasmic reticulum. *J. Biol. Chem.* **265:** 10813-10899.

Mazzarella, R.A. and M. Green, 1987. ERp99, an abundant conserved glycoprotein of the endoplasmic reticulum, is homologous to the 90-KDa heat shock protein (hsp90) and the glucose-regulated protein (grp94). *J. Biol. Chem.* **262:** 8875-8883.

Melnick, J., J.L. Dul and Y. Argon, 1994. Sequential interaction of the chaperones BiP and grp94 with immunoglobulin chains in the endoplasmic reticulum. *Nature* **370:** 373-375.

Mizzen, L.A., C. Chang, J.I. Garrels, and W.J. Welch, 1989. Identification, characterization and purification of two mammalian stress proteins present within mitochondria, grp75, a member of the hsp70 family and hsp58, a homolog of the bacterial groEL protein. *J. Biol. Chem.* **264:** 20664-20675.

Munro, S. and R.B. Pelham, 1986. An hsp70-like protein in the ER: identity with the 78kd glucose-regulated protein and immunoglobulin heavy chain binding protein. *Cell* **46:** 291-300.

Navarro D., I. Qadri, L. Pereira, 1991. A mutation in the ectodomain of herpes simplex virus 1 glycoprotein B causes defective processing and retention in the endoplasmic reticulum. *Virology* **184:** 253-264.

Picard, D., B. Khursheed, M.J. Garabedian, M.G. Fortin, S. Lindquist and K.R. Morimoto, 1990. Reduced levels of hsp90 compromise steroid receptor action *in vitro*. *Nature* **348:** 166-168.

Poussegur. J., R.P.C. Shiu, and I. Pastan, 1977. Induction of two transformation sensitive membrane polypeptides in normal fibroblast by a block in glycoprotein synthesis or glucose deprivation. *Cell* **11:** 941-947.

Sanchez, E.R., M. Hirst, L.C. Scherrer, H.Y. Tang, M.J. Welsh, J.M. Harmon, S.S. Simons, Jr., G.M. Ringold, and W.B. Pratt, 1990. Hormone-free mouse glucocorticoid receptors overexpressed in Chinese Hamster Ovary cells are localized to the nucleus and are associated with both hsp70 and hsp90. *J. Biol. Chem.* **265:** 20123-20130.

Sargan, D.R., M.J.T. Tsui, and B. W. O'Malley, 1986. Hsp108, a novel heat shock inducible protein of chicken. *Biochem.* **25:** 625-629.

Towbin, H., T. Stachelin, and J. Gordon, 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci.* **76:** 4350-4354.

Vaux D., J. Tooze, and S. Fuller, 1990. Identification by anti-idiotype antibodies of an intracellular membrane protein that recognizes a mammalian endoplasmic reticulum retention signal. *Nature* **345:** 495-502.

Welch, W.J., H.S. Kang, R.P. Beckmann, and L.A. Mizzen, 1991. Response of mammalian cells to metabolic stress; changes in the cell physiology and structure/function of stress proteins. *Curr. Topics in Microbiol. and Immunol.* **167:** 30-55.

Welch, W.J., J.I. Garrels, G.P. Thomas, J.J. Lin, and J. R. Feramisco, 1983. Biochemical characterization of the mammalian stress protein and identification of two stress proteins as glucose and Ca^{2+} ionophore regulated proteins. *J. Biol. Chem.* **258:** 7102-7111.

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Grp78/BiP과 Grp94의 생화학적 분석

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Glucose-regulated proteins(grp's) 즉 grp94와 grp78/BiP은 일종의 스트레스 단백질로 tunicamycin과 Ca^{2+} ionophore를 비롯한 다양한 스트레스성 물질에 의해 합성이 증가한다. Grp78/BiP은 분비성 단백질과 막단백질의 folding과 assembly를 조절하는 molecular chaperone 기능을 수행하는 것으로 알려져 있으나 grp94의 기능은 잘 알려져 있지 않다. 본 연구는 grp's의 세포내 분포와 grp94의 기능에 대해 조사하였다. Grp's는 소포체에 있으며 C-말단의 특이 서열인 Lys-Asp-Glu-Leu이 ER retention signal로 작용한다. 그러나 Ca^{2+} ionophore에 의해 세포내 Ca^{2+} homeostasis가 파괴되었을때 grp's가 소포체에 머무는지 세포밖으로 분비되는지 논란의 대상이 되고있다. 본 연구 결과 Ca^{2+} ionophore 존재하에서, grp94와 grp78/BiP는 세포내 특히 소포체에 위치하는 것으로 나타났다. 그리고 grp94가 molecular chaperone으로 작용하는지 조사한 결과, 항체를 분비하는 하이브리도마 세포에서 grp94가 당화된 immunoglobulin heavy chain과 일시적으로 결합하는 것으로 나타났다. 따라서 grp94는 항체의 folding과 assembly과정을 조절하는 molecular chaperone으로 작용하는 것으로 사료된다.