BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **232,** 26–32 (1997)
ARTICLE NO. RC976215

Protein Glycosylation in a Heat-Resistant Rat Fibroblast Cell Model Expressing Human HSP70

Kurt J. Henle,*[,1] Sunita M. Jethmalani,* Ligeng Li,† and Gloria C. Li†
**Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205-5484; and*
†Departments of Medical Physics and Radiation Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Received January 27, 1997

Thermotolerance and heat resistance are frequently associated with elevated levels of heat shock proteins (HSPs). Elevated heat resistance is also found to be associated with the overexpression of high levels of HSP70, as seen in M21 cells, derived from the Rat-1 line. In the present study, we report that M21 cells also feature an increase in general protein glycosylation and specific expression of the stress glycoprotein, GP62, both of which correlate with cellular heat resistance. The expression of GP50, a major stress glycoprotein in cell lines such as CHO, however, did not correlate with cellular heat resistance in M21 cells. Protein glycosylation that occurs during acute heat stress ("prompt" glycosylation) was associated with the glycosylation of a major "prompt" stress glycoprotein, P-SG64 (M_r of 64,000), that was identified by immunoblotting as a glycosylated form of calreticulin. The higher level of protein glycosylation in M21 cells correlated well with increased D-[2-^{3}H]mannose incorporation into precursor pools of dolichyl phosphomannose and dolichyl pyrophosphoryl oligosaccharides and into glycoproteins. Thus, heat resistance in M21 cells is associated not only with expression of high levels of HSP70, but also with a concomitant increase in protein glycosylation. These data support the hypothesis that stress-induced protein glycosylation is a component of cellular stress response, either in association with HSPs or as an independent mechanism.

Exposure of eukaryotic cells to heat, or to other forms of cellular stress results in the elevated expression of heat shock proteins (HSP) [1, 2] and development of cellular thermotolerance [3]. However, many cell lines also exhibit enhanced glycosylation of certain inducible proteins concurrently with increased HSP expression [4-9]. In CHO cells, the specific glycosylation of a 50-kDa glycoprotein, GP50, was seen to correlate with both expression of thermotolerance [5, 10] and accumulation of major HSPs [7]. GP50 is the homolog of a retinoic acid-inducible J6 gene product [11] and a member of the serpin family of proteins [12]. Members of the serpin superfamily have multiple regulatory functions, ranging from cell differentiation to protein folding and tumor suppression [13].

Acute heat-stress causes glycosylation of two major "prompt" stress glycoproteins (P-SG) immediately during heat stress; in CHO cells [8], both of these were identified as glycosylated forms of calreticulin [14, 15]. Together, these results indicate that the cellular stress response has a significant glycobiological component that may participate in protecting cells against stress-related damage [16]. However, a specific functional role for stress glycoproteins and their relationship to HSPs remains to be determined.

Recently, we reported the appearance of a novel stress glycoprotein, GP62, in a cell line characterized by stable overexpression of human HSP70 [17-19]. This cell line, designated as M21, is derived from Rat-1 cells, and is marked by increased heat resistance. GP62 was partially characterized and found to be a homolog of HSP70 [19]. In the present study, we specifically sought - 1) to determine GP50 vs. GP62 expression and glycosylation [5, 10] during thermotolerance expression when cells recover from initial thermal damage, and 2) to determine the "prompt" heat stress response, i.e., glycosylation of calreticulin during acute heat stress [14, 15] in M21 vs. Rat-1 cells. The data show that enhanced heat resistance in M21 cells and the constitutive overexpression of human HSP70 also en-

[1] To whom correspondence and reprint requests should be addressed at: Medical Research 151, VA Medical Center, 4300 W 7th Street, Little Rock, AR 72205-5484. Fax: (501) 671-2510; E-mail: KJHenle@Life.UAMS.edu.

Abbreviations: CHO, Chinese hamster ovary cells; Dol-P-Man, dolichyl phosphomannose; Dol-P-P- oligosaccharide, dolichyl pyrophosphoryl oligosaccharide; Endo H, endoglycosidase H; HSP, heat shock protein; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; P-SG, prompt stress glycoproteins; PVDF, polyvinylidene difluoride; WGA, wheat germ agglutinin.

0006-291X/97 $25.00

hanced protein glycosylation which may be a significant co-factor in the expression of cellular stress tolerance.

MATERIALS AND METHODS

Cell culture, heating, and materials. Rat-1 and M21 cells were grown in DMEM medium, supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), as previously reported [17, 18]. Cells were subcultured twice weekly to maintain cultures in an exponential growth pattern. For heat treatments, cells were horizontally submersed in well circulated, precision-controlled ($< \pm 0.05°$) water baths [6, 8]. D-[2-^{3}H]mannose (specific activity, 23 Ci/mmol) was purchased from ICN Radiochemicals (Irvine CA); [α-^{32}P]dCTP was from NEN Research Products (Boston, MA); polyvinylidene difluoride (PVDF) and Zeta probe nylon membranes and Bio-Spin 30 columns were from BioRad Laboratories (Hercules, CA). For Northern analyses, J6 cDNA probe was a gift from Dr. S.Y. Wang (SUNY, Albany, NY) and the hsp70 cDNA probe was obtained from StressGen Biotechnologies Corp., (Victoria, B.C., Canada). For Western blotting, a polyclonal rabbit J6 antibody (used at 1:500 dilution) was a gift from Dr. S.Y. Wang (SUNY, Albany, NY); a monoclonal mouse anti-HSP70 (used at 1:500 dilution) was from StressGen Biotechnologies Corp., (Victoria, B.C., Canada). The polyclonal (rabbit) anti-calreticulin antibody (used at 1:500 dilution) was obtained from Affinity BioReagents, Inc. (Neshanic Station, NJ). Secondary goat anti-rabbit and anti-mouse antibodies linked to alkaline phosphatase (BioRad Laboratories, Hercules, CA) were used to identify GP50/J6, calreticulin and HSP70.

Cell labeling. Cells were labeled with 50 μCi/ml of D-[2-^{3}H]-mannose, as previously described [6, 10, 19]. Typically, both control and heat-stressed cells were labeled for 1 h at 37°C. For thermotolerant cells, labeling was initiated 5 h following 15 min of 45°C-hyperthermia. For labeling of "prompt" glycoproteins [8], D-[2-^{3}H]mannose was added 1 min prior to heating for 30 min at 45°C; control cells were labeled in parallel for 30 min at 37°C. Cells were further processed as described [6, 8, 10]. Protein concentration was measured by the Coomassie Blue dye method (Bio-Rad protein assay kit) using bovine serum albumin as a standard.

Electrophoresis, fluorography and Western blotting. One-dimensional SDS-PAGE or two-dimensional electrophoresis was performed as described earlier [6, 8]. Gel lanes were loaded for equal protein, or equal radioactivity. After electrophoresis, gels were stained and soaked in En^{3}Hance (NEN Products, Boston, MA), dried, and exposed to X-Omat film (Eastman Kodak) at -70°C up to 60 days. In some experiments, D-[2-^{3}H]mannose-labeled proteins were electroblotted to PVDF membranes following electrophoresis. Relevant proteins were identified as stress-induced glycoproteins by overlay of blots with fluorograms of the same membranes, some of which were sprayed with En^{3}Hance before exposure to film. Fluorograms were analyzed by laser densitometry (Computing densitometer, Model 300A, Molecular Dynamics, Sunnyvale, CA), as described earlier [6, 8, 15]. For immunoblotting, respective antibodies were used at dilutions mentioned above, and blots were developed using the Amplified Alkaline Phosphatase Immuno-Blot assay kit from Bio-Rad (Hercules, CA) [20].

Northern blot analysis. Total RNA was extracted with guanidinium thiocyanate and separated on cesium chloride gradients, as described [21, 22]. Total RNA (10 μg) was subjected to electrophoresis on 2% formaldehyde-agarose gels, and transferred to a Zeta probe nylon membrane by capillary blotting [11]. The cDNA probes used for Northern analysis were as follows - (a) J6: A 1.54 kb Eco RI fragment of mouse cDNA excised from plasmid pcG6-pBS-sk- was used to detect GP50/J6 mRNA [11, 12]. (b) HSP70: A 4.01 kb fragment of human cDNA excised from plasmid pUC8 was used to detect HSP70 mRNA. Both these cDNA probes were labeled with 50 μCi [α-^{32}P]dCTP to 1-4 $\times$ 10^6 cpm/ng using the Multi-Prime labeling system (Amersham Corp., Arlington Heights, IL) and then purified using Bio-Spin 30 columns. Blots containing transferred RNA were hybridized with labeled probes, washed and exposed to film [11]. Relative mRNA levels were quantitated by densitometry following normalization with 28S ribosomal RNA, as described [22].

D-[2-^{3}H]mannose incorporation into Dol-P-Man, lipid-linked oligosaccharides, and glycoproteins. Cells were labeled with 6.6 μCi/ml of D-[2-^{3}H]mannose using the conditions described above. At the end of respective incubations, cells were washed, scraped, sonicated, and protein concentration determined as mentioned above. D-[2-^{3}H]-mannose incorporation into Dol-P-Man, lipid-linked oligosaccharides and glycoproteins was determined as described [23, 24]. Samples (75 μl) were added to 0.5 ml of $CHCl_3/CH_3OH$ (2:1) and 0.15 ml of water in Eppendorf tubes, thoroughly mixed, and centrifuged after 10 min at room temperature; the lower organic phase was removed and saved. The upper layer and the interface were extracted once more with 0.5 ml of $CHCl_3/CH_3OH$ (2:1). The lower phase was removed and pooled with the previously saved lower phase. The pooled organic phase was washed with 0.5 ml each of 0.9% saline and 0.9% saline/CH_3OH (1:0.5), as described [23]. In each case, the aqueous phase was separated by centrifugation and discarded. The organic phase was transferred to scintillation vials, dried, and counted using 10 ml of Instagel XF liquid scintillation cocktail (Packard Instruments Co. Inc., IL).

Acetone was added to aqueous phase and cell pellet remaining from 2:1 extraction in amount sufficient to obtain a single phase. A pellet was isolated again by centrifugation, washed thrice with 0.5 ml of water, and extracted thrice with $CHCl_3/CH_3OH/H_2O$ (1:1:0.3). Combined 1:1:0.3 extracts were transferred to scintillation vials, dried, and counted using 10 ml of Instagel XF. Pellets remaining after $CHCl_3/CH_3OH/H_2O$ (1:1:0.3) extraction were dissolved by boiling in 0.5 ml of 1% SDS, transferred to scintillation vials, and counted using 10 ml Instagel XF. The above extraction procedure results in separation of dolichyl phosphomannose (Dol-P-Man, soluble in $CHCl_3/CH_3OH$, 2:1), and dolichyl pyrophosphoryl oligosaccharide (Dol-P-P-oligosaccharide, soluble in $CHCl_3/CH_3OH/H_2O$, 1:1:0.3), while glycoproteins remain insoluble in these solvents [23].

N-glycosidase, endoglycosidase H digestions and interaction between HSP70 and GP62. D-[2-^{3}H]mannose labeled GP62 in total cell lysates was subjected to either N-glycosidase F to cleave oligosaccharide chains at the asparagine residue, or endoglycosidase H (Endo H) to cleave primarily N-linked high-mannose type oligosaccharide chains [6, 25]. Briefly, D-[2-^{3}H]mannose-labeled total protein samples (117 μg) from control and thermotolerant M21 cells were digested with either N-glycosidase F (5.4 U) or endoglycosidase H (27 mU) overnight ($\sim$16-18 h) with gentle shaking at 37°C; reactions were stopped with the addition of 3$\times$ Laemmli's buffer [25]. Samples were subjected to SDS-PAGE; proteins were electroblotted onto membranes and Western blotting was performed as described above.

For determination of interactions between HSP70 and GP62, D-[2-^{3}H]mannose labeled GP62 was subjected to WGA chromatography as described [19], with the exception of an additional step where the column was washed with 15 ml of buffer A (50 mM Tris, pH 7.5, 200 mM NaCl, 1 mM $CaCl_2$, 1mM $MnCl_2$, 7mM $MgCl_2$, 0.2 mM phenylmethylsulfonly fluoride, 0.5 μg/ml leupeptin and 0.5 μg/ml pepstatin, and 0.1% Nonidet P-40) containing 5 mM ATP. The column was then washed with buffer A containing only 1mM $MgCl_2$, and glycoproteins were eluted with N-acetyl-D-glucosamine as described [19]. The respective fractions collected were concentrated [19] and subjected to SDS-PAGE; proteins were electroblotted and Western blotting was performed as described above.

RESULTS AND DISCUSSION

In this study, we examined stress-induced protein glycosylation in M21 cells that are characterized by

overexpression of human HSP70 and increased heat resistance [17]. The data presented here show that the elevated expression of the human HSP70 was paralleled by increased glycosylation of the stress glycoproteins, GP62 and P-SG64. GP62 is known to be a partial homolog of HSP70 and cross-reacts with antibodies to HSP70 [19], whereas P-SG64 is a glycosylated variant of calreticulin [15].

Characterization of GP62 and GP50 Glycosylation during Thermotolerance Expression

Fig. 1 demonstrates that GP62 exists as two isoforms with pI values of 5.86 and 5.94, respectively, in both control and thermotolerant Rat-1 and M21 cells. The basic isoform is more abundant and constitutively present in both Rat-1 and M21 cells. However, after thermotolerance induction, D-[2-^{3}H]mannose incorporation is significantly increased in the acidic form in Rat-1 cells, and in the basic form in M21 cells.

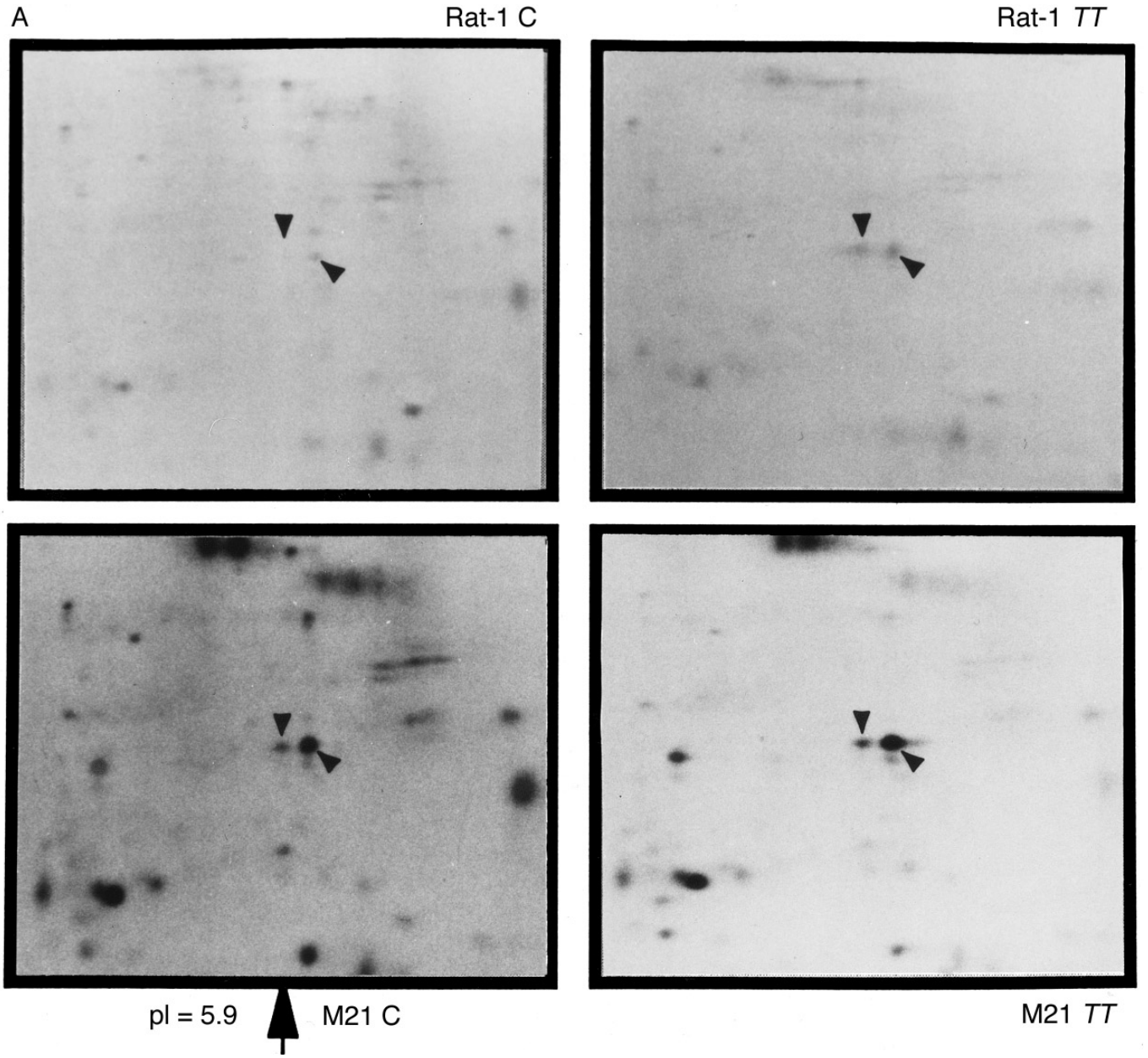
The nature of the glycosylated moieties on GP62 was investigated by treatment of D-[2-^{3}H]mannose-labeled thermotolerant cell samples with Endo H and N-glycosidase F. All glycoproteins including GP62 were deglycosylated, as indicated by the fluorogram (data not shown). Western blotting with an anti-HSP70 antibody to detect GP62 after treatment with N-glycosidase, showed reduced cross-reactivity of the GP62 band with the anti-HSP70 antibody (Fig. 1B). This suggests that GP62 was deglycosylated to a lower molecular weight protein. In contrast, treatment with Endo H did not have a significant effect on the GP62 band (Fig. 1B); this suggests that the N-linked carbohydrate moieties of GP62 are comprised predominantly of complex-type oligosaccharides, rather than high-mannose type oligosaccharides.

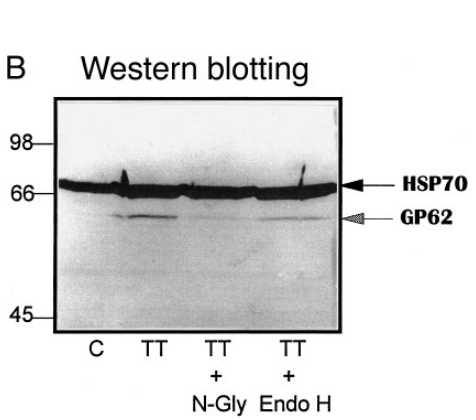
Previous studies of heat stress-induced protein glycosylation focused on GP50, a stress glycoprotein that correlates with the expression of thermotolerance in CHO cells [16]. In the present study, we found that GP62 glycosylation correlates with cellular heat resistance in both Rat-1 and M21 cells, in spite of higher GP50 glycosylation in Rat-1 cells compared to the more heat-resistant M21 cells (Fig. 2A). A similar reciprocal relationship of GP50 expression with heat resistance in Rat-1 *versus* M21 cells was also documented in studies designed to measure changes in J6/GP50 mRNA expression (Fig. 2B). These studies confirm the strong heat inducibility of J6/GP50 mRNA in CHO cells reported earlier [11], and show a progressively lower magnitude of J6/GP50 mRNA induction in Rat-1 and M21 cells, respectively (Fig. 2B). The J6/GP50 mRNA induction by heat was compared with that for HSP70. The Northern blot (Fig. 2C) confirms the presence of the higher molecular weight constitutive human (h) HSP70 message and clearly resolves the endogenous HSP70 mRNA into an inducible (i) and constitutive (c) forms of differing molecular weights [17]. These results are also consistent with earlier reports [26] that hsc70 was expressed at control temperatures and not significantly induced by heat stress, in contrast to hsp70 which was mostly induced by heat stress. A comparison of Fig. 2B with Fig. 2C indicates a more rapid relative maximum for HSP70 mRNA induction and slower kinetics for J6/GP50 mRNA induction, relative to those for endogenous HSP70, especially in rat cell lines. The relative abundance of GP50 message is further demonstrated by Western blotting (Fig 2D), where Rat-1 cells show higher GP50 levels than either M21 or CHO cells. Here, as in Fig. 2B, the heat inducibility is again minimal for Rat-1 cells. The presence of a second protein band in thermotolerant CHO cells (Fig. 2D, lane 2) appears to be a variable, glycosylated form of GP50. Overall, the data consistently demonstrate GP62 as the major heat stress-induced glycoprotein in the rat cell lines, particularly in the HSP70-overexpressing M21 cells. In contrast, CHO cells exhibit GP50-associated thermotolerance after appropriate heat conditioning.

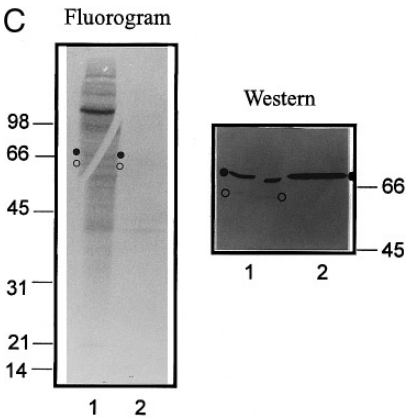
"Prompt" Glycosylation

Acute heat stress mediates a "prompt" glycosylation phenomenon [8, 16] that differs from the "late" glyco-

FIG. 1. Identification of GP62 isoforms, characterization of glycosylated moieties of GP62 and interaction between HSP70 and GP62. Panel A, Characterization of GP62 by two-dimensional electrophoresis. The four separate panels show corresponding portions of fluorograms from two-dimensional gels resolving D-[2-^{3}H]mannose-labeled proteins from Rat-1 and M21 cells. Gel lanes were loaded for equal protein (80 μg) to show relative labeling of the two GP62 isoforms. Triangular arrows show the isoforms of GP62 having similar molecular weight and distinct pI values near pI=5.9. Panel B, Treatment of GP62 with N-glycosidase F and Endoglycosidase H and its identification by cross-reactivity with anti-HSP70 antibody. Abbreviations: C, control unstressed M21 cells; TT, thermotolerant M21 cells without any glycosidase digestions; TT+N-gly, digestion with 5.4 U of N-glycosidase F, TT+Endo H, digestion with 27 mU of Endo H. Panel C, Interaction between HSP70 and GP62 by affinity binding using WGA-Sepharose chromatography [19]. Bound proteins were eluted with 1M N-acetyl-D-glucosamine as described in "Materials and Methods". A portion of the Western blot (probed with HSP70 specific antibodies) and the fluorogram of the same membrane are shown. Lane 1, proteins eluted with 1M N-acetyl-D-glucosamine following ATP elution, lane 2, proteins eluted with ATP. The discontinuity observed in lanes 1 and 2 was due to gel breaking that occured during the electroblotting procedure. Molecular weight markers are shown on left and right sides. GP62 position is indicated by open circles and HSP70 position (above the glycoprotein in the fluorogram) is indicated by closed circles. Although most of HSP70 eluted with ATP, some HSP70 still co-eluted with the glycoproteins in the fraction eluted with 1M N-acetyl-D-glucosamine. These results suggest that HSP70 did not bind WGA directly, but via interactions with GP62 and/or other glycoproteins; total elution of HSP70 may be possible by varying the conditions for ATP elution.


A
Rat-1 C
Rat-1 TT
pI = 5.9
M21 C
M21 TT


B
Western blotting
98
66
45
HSP70
GP62
C
TT
TT + N-Gly
TT + Endo H


C
Fluorogram
98
66
45
31
21
14
1
2
Western
66
45
1
2

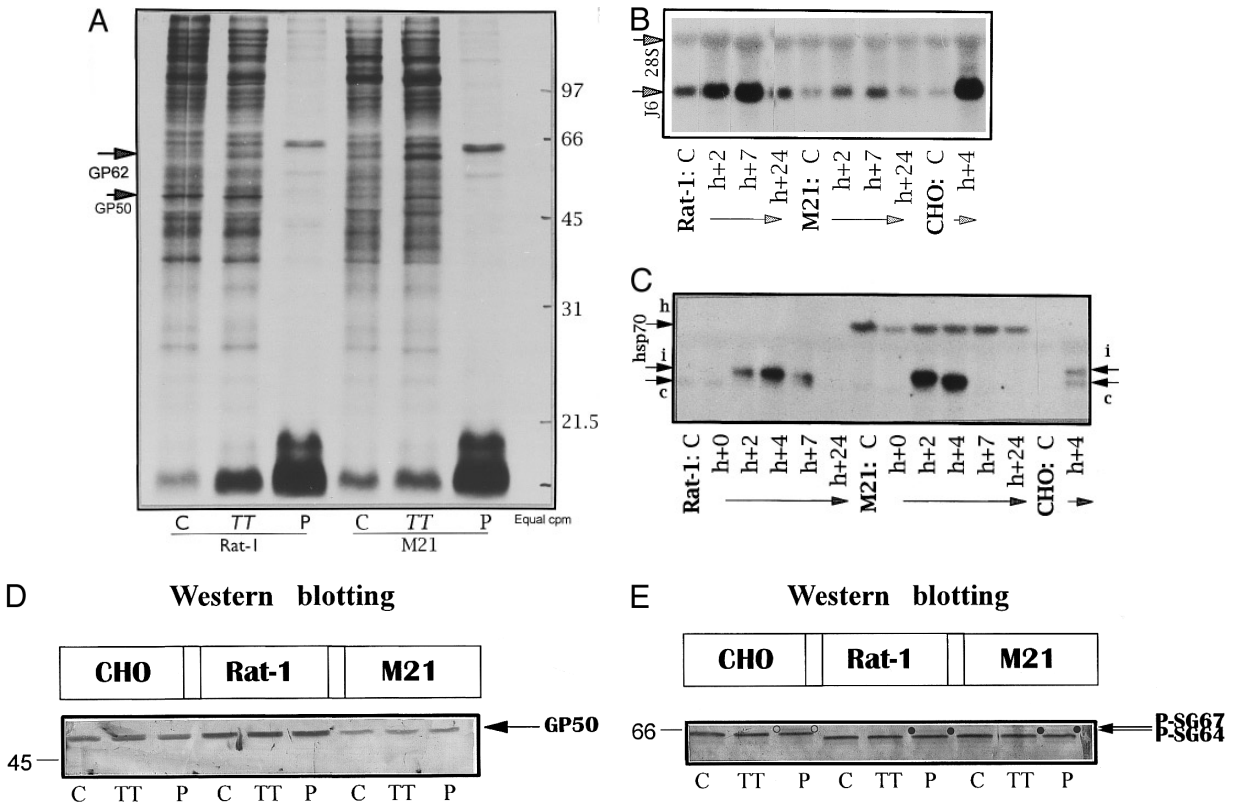


FIG. 2. Expression of stress glycoproteins in Rat-1 and M21 cells vs. CHO cells. Panel A, Fluorogram showing labeled glycoproteins in M21 and Rat-1 cells on 1D gels. Gel lanes were loaded for equal radioactivity (80,000 dpm). Panel B shows gp50/J6 mRNA expression and Panel C shows the distinct hsp70 mRNA species: h: human hsp70 mRNA; c: constitutive endogenous hsp70 mRNA (hsc); i: inducible endogenous hsp70 mRNA. Panel D, Immunoblot identifying GP50 and Panel E, Immunoblot identifying the prompt stress glycoprotein, P-SG64 in Rat-1 and M21 cells. Glycoproteins were labeled with D-[2-^{3}H]mannose and resolved by SDS-PAGE. Gel lanes were loaded for equal protein (30 μg for GP50 blot and 60 μg for P-SG64). Proteins were electroblotted to PVDF membranes that were probed with the respective antibodies. Open circles show the position of glycosylated P-SG67; dots show positions of glycosylated P-SG64; the glycosylated status of the proteins was identified by alignment with the corresponding fluorogram (data not shown). The positions of the glycoproteins is also indicated by arrows. The majority of calreticulin proteins were unglycosylated and migrated at lower molecular weights. Notations: C, control cells (no heating); h+0, h+2, h+4, h+7 and h+24 denotes heat stress for 15 min (rat cells) or 10 min (CHO cells) at 45°C followed by recovery periods from 0-24 h; TT denotes thermotolerant cells; P denotes prompt heat-stress (heat-stress- 30 min at 45°C).

sylation discussed above (Fig. 2A). In CHO cells, the major prompt stress glycoproteins, P-SG67 and P-SG64 (M_r of 64,000) are two different isoforms/glycoforms of the same protein [14, 15] and are not converted into other stress glycoproteins with lower molecular weight, e.g., GP62 or GP50 [8, 16]. In Rat-1 and M21 cells, "prompt" heat-stress (30 min at 45°C) resulted in the selective glycosylation primarily of P-SG64 (Fig. 2A, lanes 3 and 6). Western blotting identified P-SG64 also as calreticulin (Fig. 2E), a protein that is abundant in both CHO and Rat-1 cells. Acute heat stress caused a small, but significant shift in the calreticulin bands towards higher molecular weights (see open circles and dots above the main band in Fig. 2E). Superimposition of the fluorogram with the blot identified these as D-[2-^{3}H]mannose-labeled P-SG64 (dots, Fig. 2E) in Rat-1 and M21 cells, and as P-SG67 (open circles, Fig. 2E) in CHO cells. The amount of calreticulin and the appearance of P-SG64 in Rat-1 and M21 cells was relatively constant, in contrast to that of GP62 and GP50, as discussed above.

Incorporation of D-[2-^{3}H]mannose into Dol-P-Man, Lipid-Linked Oligosaccharides, and Glycoproteins

Heat-induced protein glycosylation may be controlled at the level of carbohydrate precursor, intermediate pools, or through specific enzyme mediated reactions [20]. Fig. 3 shows the distribution of D-[2-^{3}H]-mannose radiolabel within precursor (Dol-P-Man and Dol-P-P-oligosaccharide) pools and glycoproteins.

Dramatic differences appeared in the labeling patterns of CHO *vs.* rat cell lines when cells were labeled with D-[2-^{3}H]mannose after thermotolerance induction. Thermotolerant CHO cells showed an approximately 90% reduction in the D-[2-^{3}H]mannose incorpo-

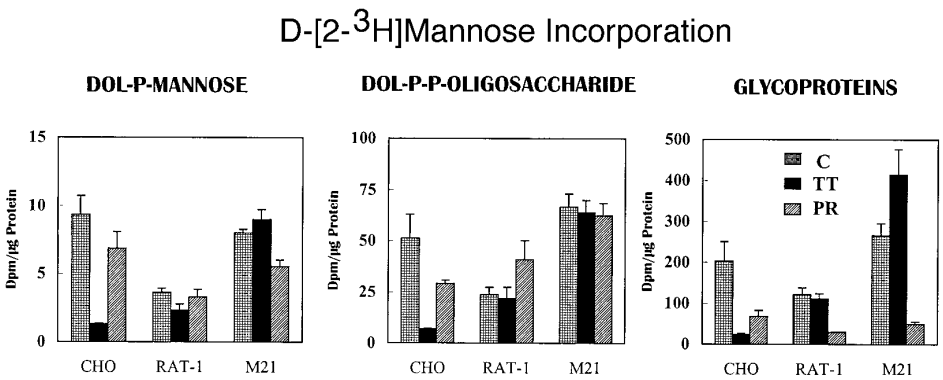


FIG. 3. D-[2-^{3}H]mannose incorporation into Dol-P-Man, Dol-P-P-oligosaccharides and glycoproteins. Cells were labeled with D-[2-^{3}H]-mannose and incorporation was determined as described in "Materials and Methods". Values represent mean ± SE; N=3 for each data point.

ration rate into both Dol-P-Man and Dol-P-P-oligosaccharide and glycoprotein fractions. On the other hand, thermotolerant Rat-1 and M21 cells showed no significant change in the D-[2-^{3}H]mannose incorporation into precursor pools. However, the D-[2-^{3}H]mannose incorporation into glycoproteins was increased by more than 30% in thermotolerant M21 cells, but remained unchanged in thermotolerant Rat-1 cells compared to unheated control cells. Acute heat stress ("prompt" glycosylation) had only modest, or negligible effects on the specific activity of D-[2-^{3}H]mannose incorporation into precursor pools of all three cell lines. Furthermore, D-[2-^{3}H]mannose incorporation into glycoproteins was significantly reduced in all three cell lines. However, the data in Fig. 2A indicate that the glycosylation occured selectively on P-SG67/64.

The above results suggest that D-[2-^{3}H]mannose incorporation into glycoproteins and its precursor pools during thermotolerance induction may be linked to a particular phenotype that utilizes either GP50 or GP62 as the major stress glycoprotein. Similarly, the selective incorporation of D-[2-^{3}H]mannose into either P-SG67 (CHO cells) and P-SG64 (Rat-1 and M21 cells) also appears to be cell line specific. Nevertheless, the data presented in this paper clearly show that glycosylation of GP62 correlates with heat sensitivity in the Rat-1 and M21 cell lines, whereas in CHO cells GP50 appears to be linked to cellular heat sensitivity.

In summary, our data show alterations in GP50, GP62 and P-SG64 glycosylation that occur in parallel with the elevated levels of HSP70 in M21 cells. Thus, stress glycoproteins, together with HSP70 must also be considered as potential contributors to increased cellular heat resistance. While GP50 is the major stress glycoprotein in CHO cells, GP62 appears to be the major stress glycoprotein in rat lines, Rat-1 and M21.

Stress-induced protein glycosylation appears to be an integral component of the cellular stress response, at least in mammalian cells [16]. The role of glycosylation remains to be defined, but is known capable of modulating protein interactions and the regulation of specific cell functions [27]. Similarly, information about the regulation of stress-mediated glycosylation is largely hypothetical. However, protein glycosylation is known to occur when proteins are unfolded and potential glycosylation sites become exposed, a process that might involve HSP chaperones [16] and the formation of protein-chaperonin complexes [28]. For example, members of the HSP60 or Cpn60 family associate as homo-oligomers into two stacked rings with sevenfold symmetry [29]. The functional folding complex may also require interaction with other stress response elements in a sequential or cooperative manner as in the case of mitochondrial protein import, where molecules are passed from a HSP70 complex to a HSP60 complex [30].

Overexpression of human HSP70 in M21 cells may, thus, mediate the overexpression of chaperone "cohorts" [31, 32], co-factors that are required for the assembly of a fully functional chaperone machinery [16]. "Prompt" stress glycoproteins may also fit into such a model, sharing similarities with other chaperones, e.g., GRP78, GRP94 and protein disulfide-isomerase [33-35]. Calreticulin, the aglycone form of P-SG67/64, is closely related to calnexin, a new type of Ca^{2+}-dependent chaperone specifically linked to stress-induced glycosylation [36]. The ATP-dependent interaction between HSP70 and at least one stress glycoprotein, GP62, is clearly evident from our studies on affinity binding of GP62 to WGA (Fig. 1C). Furthermore, our immunoprecipitation studies also indicate similar interactions between HSP70 and GP62 (manuscript in preparation), but not between HSP70 and P-SG64/calreticulin [37]. Future studies will determine whether glycosylation functions as an independent, or as a complementary mechanism to the known roles of classical HSPs.

ACKNOWLEDGMENTS

This investigation was supported in part through PHS grant number CA-33405 (to K.J.H.) and CA-56909 (to G.C.L.), awarded by the

National Cancer Institute, DHHS. The authors thank Mr. Greg T. Nolen and Mrs. Virginia A. Johnson for excellent technical support.

REFERENCES

1. Schlesinger, M. J. (1994) *Pediatr. Res.* **36,** 1–6.
2. Parsell, D. A., and Lindquist, S. (1993) *Annu. Rev. Genet.* **27,** 437–496.
3. Henle, K. J., and Dethlefsen, L. A. (1978) *Cancer Res.* **38,** 1843–1851.
4. Henle, K. J. (1987) *in* Thermotolerance (Henle, K. J., Ed.), Vol. 2, pp. 13–71, CRC Press, Inc., Boca Raton, FL.
5. Henle, K. J., Norris, J. S., Nagle, W. A., and Moss, A. J. (1988) *Int. J. Radiat. Biol.* **53,** 839–847.
6. Henle, K. J., and Nagle, W. A. (1991) *Exp. Cell Res.* **196,** 184–191.
7. Henle, K. J., and Nagle, W. A. (1992) *J. Cell. Biochem.* **16D,** 163.
8. Henle, K. J., Kaushal, G. P., Nagle, W. A., and Nolen, G. T. (1993) *Exp. Cell Res.* **207,** 245–251.
9. Lee, Y. J., Kim, D., Hou, Z.-Z., and Corry, P. M. (1991) *J. Cell. Physiol.* **149,** 202–207.
10. Henle, K. J., Nagle, W. A., Bedford, J. S., and Harvey, W. F. (1990) *J. Cell Sci.* **95,** 555–561.
11. Henle, K. J., Wang, S.-Y., Nagle, W. A., and Lumpkin, C. K. (1994) *Exp. Cell Res.* **210,** 185–191.
12. Wang, S.-Y., and Gudas, L. J. (1990) *J. Biol. Chem.* **265,** 15818–15822.
13. Potempa, J., Korzus, E., and Travis, J. (1994) *J. Biol. Chem.* **269,** 15957–15960.
14. Jethmalani, S. M., Henle, K. J., and Kaushal, G. P. (1994) *J. Biol. Chem.* **269,** 23603–23609.
15. Jethmalani, S. M., and Henle, K. J. (1994) *Biochem. Biophys. Res. Commun.* **205,** 780–787.
16. Henle, K. J., Jethmalani, S. M., and Nagle, W. A. (1995) *Trends Glycosci. Glycotechnol.* **7,** 191–204.
17. Li, G. C., Li, L., Liu, Y.-K., Mak, J. Y., Chen, L., and Lee, W. M. F. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1681–1685.
18. Li, G. C., Li, L., Liu, Y.-K., Rehman, M., and Lee, W. M. F. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2036–2040.
19. Jethmalani, S. M., and Henle, K. J. (1997) *Exp. Cell Res.,* In press.
20. Henle, K. J., Stone, A., and Chatterjee, S. K. (1988) *Cancer Res.* **48,** 5717–5721.
21. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Vol. I, pp. 7.23–7.29, Cold Spring Harbor Lab., Cold Spring Harbor, NY.
22. Badger, T. M., Ronis, M. J. J., Lumpkin, C. K., Valentine, C. R., Shahare, M., Irby, D., Huang, J., Mercado, C., Thomas, P., Ingelman-Sundberg, M., and Crouch, J. (1992) *J. Pharmacol. Exp. Therap.* **264,** 438–447.
23. Tabas, I., Schlesinger, S., and Kornfeld, S. (1978) *J. Biol. Chem.* **253,** 716–722.
24. Chambers, J., and Elbein, A. D. (1975) *J. Biol. Chem.* **250,** 6904–6915.
25. Dorner, A. J., and Kaufman, R. J. (1990) *Methods Enzymol.* **185,** 577–596.
26. Lindquist, S., and Craig, E. A. (1988) *Annu. Rev. Genet.* **22,** 631–677.
27. Dwek, R. A. (1995) *Science* **269,** 1234–1235.
28. Ellis, R. J., and Van der Vies, S. M. (1991) *Annu. Rev. Biochem.* **60,** 321–347.
29. Hendrick J. P., and Hartl, F.-U. (1993) *Annu. Rev. Biochem.* **62,** 349–384.
30. Becker, J., and Craig, E. A. (1994) *Eur. J. Biochem.* **219,** 11–23.
31. Kelley, W. L., and Georgopoulos, C. (1992) *Curr. Opin. Cell Biol.* **4,** 984–991.
32. Georgopoulos, C. (1992) *Trends Biochem. Sci.* **17,** 295–299.
33. Nigam, S. K., Goldberg, A. L., Ho, S., Rohde, M. F., Bush, K. T., and Sherman, M. Y. (1994) *J. Biol. Chem.* **269,** 1744–1749.
34. Wada, I., Imai, S.-I., Kai, M., Sakane, F., and Kanoh, H. (1995) *J. Biol. Chem.* **270,** 20298–20304.
35. Nauseef, W. M., McCormick, S. J., and Clark, R. A. (1995) *J. Biol. Chem.* **270,** 4741–4747.
36. Bergeron, J. J. M., Brenner, M. B., Thomas, D. Y., and Williams, D. B. (1994) *Trends Biochem. Sci.* **19,** 124–128.
37. Jethmalani, S. M., and Henle, K. J. (1997) *J. Cell. Biochem.* submitted.