Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 319 (2004) 21–26
BBRC
www.elsevier.com/locate/ybbrc

70-kDa-heat shock protein presents an adjustable lectinic activity towards O-linked *N*-acetylglucosamine

Céline Guinez, Jérôme Lemoine, Jean-Claude Michalski, and Tony Lefebvre*

UMR 8576 du CNRS, IFR 118, Unité de Glycobiologie Structurale et Fonctionnelle, Bâtiment C9, Cité Scientifique, 59655 Villeneuve d'Ascq cedex, France

Received 21 April 2004
Available online 10 May 2004

Abstract

Numerous works demonstrated that the dynamic *O*-GlcNAc glycosylation could protect against the proteasomal degradation by modifying the target proteins and the proteasome itself. Considering that Hsp70 is a crucial component in the quality control of protein conformation in the proteasomal pathway, we investigated the possibility that Hsp70 physically interacts with *O*-GlcNAc proteins through a lectinic activity. First, we demonstrate that in HepG2 cells, Hsp70 can specifically bind to *O*-GlcNAc residues but also is itself modified by *O*-GlcNAc. Second, when cells were deprived of glucose (nutrient stress), Hsp70 lectinic activity markedly increased whereas its glycosylation dramatically decreased. On the other hand, a 42 °C thermic stress did not affect any of these features. Lastly, the nature of *O*-GlcNAc modified proteins co-immunoprecipitating with Hsp70 was similar for cells submitted to the thermic and to nutrient stress. These results strongly suggest that *O*-GlcNAc influences protein stability through specific interaction with 70-kDa-heat shock protein members.

Keywords: Heat shock proteins; Hsp70; Chaperone; *O*-GlcNAc; Glucose; Stress

Usually, the proteins targeted to the proteasome present PEST sequences (sequences enriched in proline, glutamic acid, serine, and threonine that direct proteins for rapid degradation [1]) and are modified by poly-ubiquitinylation (for review see reference [2]). Recent reports showed that the dynamic *O*-GlcNAc glycosylation of proteins within the cytosolic and nuclear compartments of eukaryotes [3], such as Sp1 [4], estrogen receptor-β [5], and more recently plakoglobin [6], was less affected by proteasomal degradation. Consequently, it has been proposed that *O*-GlcNAc could protect proteins from the proteasomal degradation. Studies performed on Sp1 have shown that in cells cultured in the presence of 5 mM glucosamine, Sp1 became hyperglycosylated whereas in glucose starvation, the glycosylation decreased and Sp1 was rapidly degraded by the proteasome [4]. Treatment of the cells with glucose or glucosamine protects Sp1 from the degradation. This process seems to be independent of ubiquitinylation process. For the β-estrogen receptor, it has been proposed that the *O*-GlcNAc glycosylation of the site found within the PEST sequence could block protein degradation and would thus played an opposite role to that play by phosphorylation (which activates degradation at PEST sequences) [5]. Recently, it was shown that plakoglobin, which connects cadherins to cytoskeleton, could be modified with *O*-GlcNAc in close proximity to the destruction box [6]. Interestingly, many of the well-characterized *O*-GlcNAc glycosylation sites show high PEST scores, reinforcing the relationship between proteasomal degradation and *O*-GlcNAc. Two recent studies showed that the 26S proteasome is also modified by *O*-GlcNAc both in the regulatory and in the catalytic cores [7,8] and that, the *O*-GlcNAc moiety acts as an inhibitor of the proteolysis function.

In a previous report, we described for the first time the lectinic activity of the Hsc70 protein, a constitutive member of the 70-kDa-heat shock protein family (HSP70) [9]. Heat shock proteins (HSPs) are ubiquitously

* Corresponding author. Fax: +33-3-20-43-65-55.
E-mail address: tony.lefebvre@univ-lille1.fr (T. Lefebvre).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.04.144

found in all organisms ranging from bacteria to most complex metazoans. They share a conserved structure that suggests a fundamental function in many cellular processes and are distributed in mostly all organelles (for review see [10]). HSP are composed of several families with molecular masses spreading from 20 to 110 kDa. The HSP70 family exhibits 70% base identity among the entire HSP70 eukaryotic family and near 60% with DnaK, its homolog in *Escherichia coli*. HSP70 are mainly localized in the cytosol and the nucleus of eukaryote cells. Their expression is induced by different types of stress (e.g., temperature, irradiation, heavy metals, infection, fever, and inflammation [11]). However, HSP70 are also present at a minimal amount in normal conditions. HSP70 are structured in three main domains [10]: the 44 kDa N-terminal domain involved in ATP hydrolysis, the central domain of 18 kDa that binds hydrophobic peptide sequence, and a 10 kDa glycine–proline rich C-terminal domain with a conserved EEVD tetrapeptide terminal sequence implicated in the mRNA translation. The constitutive form of HSP70, named Hsc70 (Heat shock cognate protein of 70 kDa), is not inducible. Both forms bind and hydrolyse ATP [12]. In conjunction with co-chaperones, heat shock proteins are involved in the protection and in the re-folding of normal and damaged proteins. When the re-folding process is unsuccessful, proteins are directed to the ubiquitin–proteasome system (UPS) for destruction [13].

Here, we have demonstrated the ability of Hsp70 to bind in a specific manner to *O*-GlcNAc residues. These results support a new type of lectinic interaction between *O*-GlcNAc modified proteins and Hsp70, related to protein life and death.

Materials and methods

Cell culture and stress treatments. HepG2 cells were cultured on dishes (diameter 100 mm) treated with 0.1% porcine gelatin (Sigma) in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% (v/v) fetal calf serum, 2 mM L-glutamine, 5 IU/mL penicillin, and 50 μg/mL streptomycin at 37 °C in a 5%-CO_2 enriched atmosphere. For glucose depletion stress cells were rinsed with 10 mL of glucose-free medium before incubation in this culture medium for 24 h. Thermal stress was applied to cells by hyperthermic conditions culturing at 42 °C in a 5%-CO_2 enriched atmosphere.

GlcNAc-beads affinity enrichment, immunoprecipitations, and co-immunoprecipitations. Before lysis, HepG2 cells were washed with cold phosphate-buffered saline (Gibco).

For lectinic activity studies, cells were lysed in a hypotonic buffer (10 mM Tris/HCl, 10 mM NaCl, 15 mM 2-mercaptoethanol, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and protease inhibitor, pH 7.2) with a Dounce homogenizer on ice [12]. Extracts were centrifuged at 20,000*g* for 30 min at 4 °C, and the supernatant was carefully recovered. Thirty microlitres of GlcNAc-labelled beads (*N*-acetyl-D-glucosamine immobilized on 6% beaded agarose with a spacer of five carbons, Sigma) was added to the supernatant and incubated for 1 h at 4 °C. Beads were then gently centrifuged and washed four times in the buffer containing 20 mM Tris/HCl, 200 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and protease inhibitor, pH 7.4.

For immunoprecipitation, cells were lysed in a lysis buffer (Tris/HCl 10 mM, NaCl 150 mM, Triton X-100 1% (v/v), sodium deoxycholate 0.5% (w/v), sodium dodecyl sulphate 0.1% (w/v), and protease inhibitors, pH 7.4) and centrifugation was performed at 20,000*g* for 30 min at 4 °C. The RL2 anti-*O*-GlcNAc antibody (Affinity BioReagent) was added to a 1:250 final dilution and the lysates were placed at 4 °C overnight. The bound proteins were recovered after addition of protein G–Sepharose (Amersham) for 1 h at 4 °C. Beads were pelleted and washed, respectively, in: lysis buffer; lysis buffer supplemented with 500 mM NaCl; lysis buffer/TNE (Tris/HCl 10 mM, NaCl 150 mM, and EDTA 1 mM) in equal volume, and finally with TNE alone.

For co-immunoprecipitation with the anti-Hsp70 polyclonal antibody (Stressgen BioReagents) the procedure was the same as described for immunoprecipitation except that HepG2 were lysed in Co-IP buffer (Tris/HCl 50 mM, NaCl 150 mM, NP-40 0.5% (v/v), and protease inhibitors, pH 8.0) and anti-Hsp70 was used at a dilution of 1:200. The bound proteins were recovered by incubation with protein A–Sepharose (Amersham) for 1 h. Four washes were performed in the Co-IP buffer with gentle shaking (four times).

SDS–PAGE and Western blotting. Proteins were separated on 10% SDS–PAGE and electroblotted onto nitrocellulose sheet. Membranes were saturated for 45 min with 5% non-fatty acid milk in TBS–Tween buffer (Tris/HCl 20 mM, NaCl 150 mM, and Tween 0.05% (v/v), pH 8.0). Anti-Hsp70 antibodies were incubated for 1 h at a dilution of 1:150,000, and RL2 anti-*O*-GlcNAc monoclonal antibodies or anti-β-catenin polyclonal antibodies (Santa Cruz Biotechnologies) were incubated overnight at 4 °C at a dilution of 1:1000. Three washes of 10 min each were performed with TBS–Tween. Anti-rabbit and anti-mouse horseradish peroxidase labelled secondary antibodies were used at a dilution of 1:10,000 for 1 h at room temperature. Three washes of 10 min each were performed with TBS–Tween and the detection was carried out using the Western lightning chemiluminescence reagents plus kit (Perkin–Elmer).

Results and discussion

Hsp70 exhibits a lectinic activity towards the O-GlcNAc motif and is itself O-GlcNAc modified

In order to test our hypothesis of a putative lectinic property of the 70-kDa-heat shock protein towards the *O*-GlcNAc motif, GlcNAc-labelled agarose beads were incubated with a protein extract obtained from the HepG2 human hepatocarcinoma cell line cultured at 37 or at 42 °C. A lectinic activity of Hsp70 toward GlcNAc is evident in HepG2 both at 37 and at 42 °C (Fig. 1A, lanes 3 and 4). The quantitative differences of Hsp70 expression at 37 and at 42 °C, both in the control lanes and in the GlcNAc-beads enriched ones, are related to its induction after thermic stress. The specificity of the lectinic property of Hsp70 is illustrated by the less intense binding of Hsp70 to chitobiose (GlcNAc β-1,4-GlcNAc) and the lack of interaction with the mannose residue at 37 °C (Fig. 1B).

We have also examined whether Hsp70 itself might be a substrate for the *O*-GlcNAc transferase (OGT). For this purpose, using the extract from HepG2 grown at 37 °C and 42 °C, *O*-GlcNAc modified proteins were

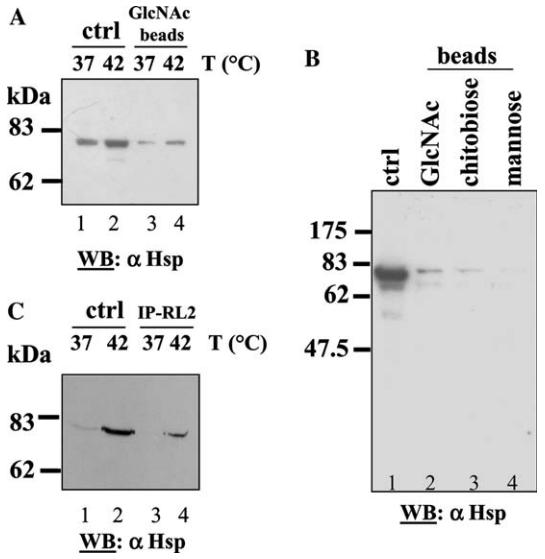

Fig. 1. Hsp70 lectinic activity against *N*-acetylglucosamine residues and *O*-GlcNAc modification of Hsp70. HepG2 cells were cultured either at a temperature of 37 °C or at a temperature of 42 °C as to induce a thermic stress. After lysis of the cells, cellular extracts were incubated either with GlcNAc-agarose beads (A) or with anti-*O*-GlcNAc monoclonal antibodies (C). The bound proteins were submitted to a 10% SDS–PAGE, electroblotted onto nitrocellulose sheet, and stained with an anti-Hsp70 antibody. The lectinic activity of Hsp70 was also tested on chitobiose beads and on mannose beads (B). The glycosylation of Hsp70 by *O*-GlcNAc was checked using the anti-*O*-GlcNAc antibody RL2 in immunoprecipitation experiments and an anti-Hsp70 antibody in Western blot (C). Ctrl, control; WB, Western blot.

enriched by immunoprecipitation with an anti-*O*-GlcNAc antibody (RL2). The bound proteins were separated on a 10% SDS–PAGE and stained with anti-Hsp70 antibodies (Fig. 1C). Indeed, Hsp70 was itself modified with *O*-GlcNAc. Only a very faint band of the glycosylated form of Hsp70 at 37 °C (Fig. 1C, lane 3) was related to the weak expression of Hsp70 at this temperature (Fig. 1C, lane 1). The increase in the GlcNAc binding and in the *O*-GlcNAc glycosylation on Hsp70 at 42 °C is attributed to its induction by the thermic stress; since in both cases the lectinic activity and glycosylation increased following the induction of Hsp70. In conclusion, this set of experiments clearly shows that Hsp70 exerts a lectinic activity towards the *N*-acetylglucosamine residues and that this protein is *O*-GlcNAc modified.

O-GlcNAc level of Hsp70 is sensitive to glucose starvation and influences its binding activity toward N-acetylglucosamine residues

UDP-GlcNAc is the sugar donor used by OGT to transfer GlcNAc residues to protein substrates. GlcNAc originates from the conversion of about 3–5% of the glucose entering the cell following the hexosamine biosynthetic pathway (Fig. 2A) [14]. As expected, the level of *O*-GlcNAc modifying proteins in cells cultured in glucose-depleted medium strongly decreased compared to cells growing in normal-culture medium (4.5 g glucose/L, Fig. 2B). Moreover, the modification of the pattern of glycosylation caused by the glucose starvation appears overall not affected by the thermic stress (Fig. 2B, compare lanes 1–3 and 2–4) except for proteins migrating between 50 and 70 kDa (arrowheads). The *O*-GlcNAc glycosylation of Hsp70 exhibits the same pattern with a similar high decrease of anti-*O*-GlcNAc staining both at 37 and at 42 °C (Fig. 2C, lanes 5–8).

Interestingly, the lectinic activity of Hsp70 in glucose-depleted conditions highly increased when cells were cultured in absence of glucose (Fig. 2D, compare lanes 5 and 6 to lanes 1 and 2). As previously shown in Fig. 1, we also observed an increase in the lectinic activity of Hsp70 after thermic stress (42 °C) that is in part related to an induction of Hsp70 (Fig. 2, lanes 1, 3, 5, and 7). This is in contrast to glucose-depleted conditions where the dramatic increase in this lectinic activity of Hsp70 is not due to a protein synthesis (Fig. 2, lanes 1, 2, 5, and 6).

These experiments demonstrate that when HepG2 cells are submitted to glucose starvation, the *O*-GlcNAc glycosylation level of Hsp70 decreases similarly to other extracted proteins but, unexpectedly, this decrease is associated with a noticeable higher lectinic affinity.

The results exposed above led us to evaluate the influence of the thermic stress (37 or 42 °C), combined with glucose starvation on the propensity of Hsp70 to associate with protein partners through interactions with the *O*-GlcNAc motif. For this experiment, anti-Hsp70 antibodies were used to co-immunoprecipitate proteins from a HepG2 cellular lysate. The co-immunoprecipitated proteins were analysed by Western blot using either the anti-*O*-GlcNAc RL2 monoclonal antibodies (Fig. 3A, top panel) or with an anti-β-catenin polyclonal antibody (Fig. 3A, bottom panel). Mainly, we can see that in the different conditions of culture (Fig. 3A, lanes 5–8), the Hsp70 co-immunoprecipitated *O*-GlcNAc protein patterns are rather similar in terms of representation except for a low molecular mass protein of 30 kDa whose co-immunoprecipitation increased in glucose-depleted conditions (Fig. 3, lanes 6 and 8). It may somewhat be intriguous to note that despite the dramatic drop in the *O*-GlcNAc rate affecting the proteins (Fig. 3A, lanes 1–4), many bands corresponding to co-immunoprecipitated *O*-GlcNAc modified proteins with Hsp70 are still detected (Fig. 3A, lanes 5–8). This phenomenon might be related to the increased affinity of the Hsp70 chaperone towards the *O*-GlcNAc, leading to a compensating behaviour with the decrease in glycosylation (Fig. 3B). When free GlcNAc was added, both to the lysate and to the washing buffer, the relative intensity of the bands of the co-immunoprecipitated proteins was lowered suggesting an obvious *O*-GlcNAc interaction between Hsp70 and these proteins

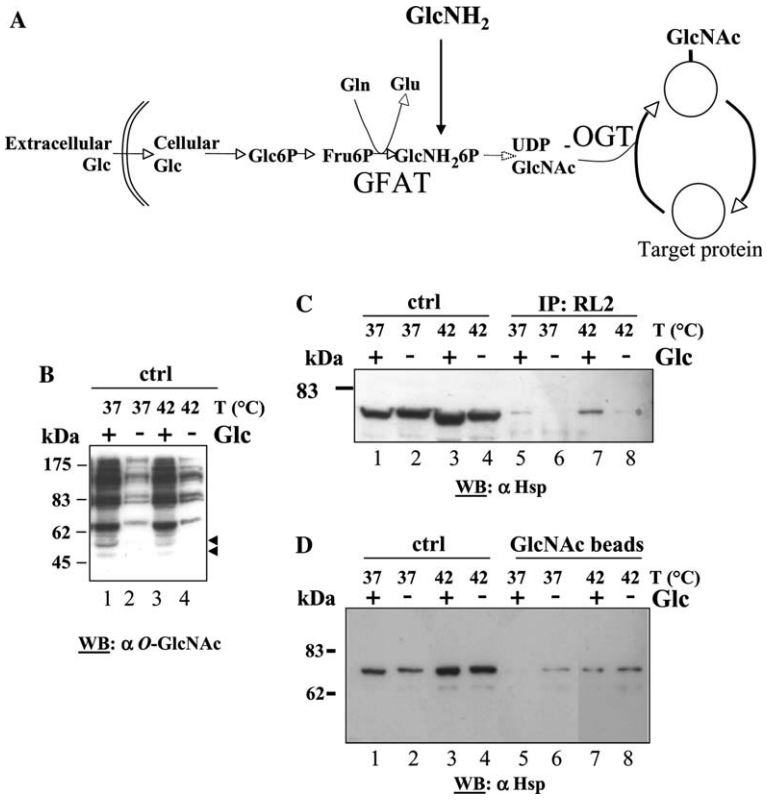

Fig. 2. Hsp70 lectinic activity towards GlcNAc and *O*-GlcNAc level in Hsp70 in HepG2 cells cultured in absence of glucose. (A) The relationship between the *O*-GlcNAc glycosylation and the extracellular glucose is drawn. Glucose enters the cell by a specific transporter, is phosphorylated to glucose 6-phosphate, and is isomerized in fructose 6-phosphate by the phosphoglucose isomerase. At this stage a key-enzyme intervenes: the glutamine-fructose amidotransferase (GFAT). It converts the fructose 6-phosphate to glucosamine 6-phosphate that is in definitive activates as a nucleotide-sugar: the UDP-GlcNAc that is the substrate of the *O*-GlcNAc transferase (OGT). The GFAT could be by-passed by treating cells with glucosamine. (B–D) Two types of stresses were applied to HepG2 cells: a thermic stress and a nutrient stress (glucose deprivation). In both cases, the lectinic activity of Hsp70 (D) and its *O*-GlcNAc glycosylation were studied (C). In the same experiment, the *O*-GlcNAc level of total cellular proteins was looked at (B) as described for Fig. 1. Blots were performed using the anti-Hsp70 antibody after running of the GlcNAc-bound proteins or the *O*-GlcNAc-enriched proteins by a 10% SDS–PAGE. Ctrl, control; T, temperature in degree celsius; IP, immunoprecipitation; and WB, Western blot.

(data not shown). However, this interaction seems to involve also the peptide moiety since free GlcNAc sugar alone is not sufficient to fully abolish this interaction. β-Catenin, that is involved in cell adhesion via interaction with E-cadherin and that plays an essential role in the Wingless/Wnt signalling pathway, has been characterized to be modified by *O*-GlcNAc [15]. Moreover, it has been demonstrated that the turnover of β-catenin is regulated by the ubiquitin–proteasome system [16]. According to the lectinic activity of Hsp70 against *O*-GlcNAc, these observations led us to strongly suppose that this key-protein could interact with Hsp70 via the *O*-GlcNAc moiety. Actually, β-catenin co-immunoprecipitated with Hsp70 (Fig. 3A, bottom panel) and this immunoprecipitation was higher when HepG2 cells were cultured in absence of glucose, i.e., when the lectinic activity of Hsp70 was maximal. In the control samples β-catenin appeared unchanged whatever the stress we applied (thermic or nutrient) and migrated as two bands, but in the co-immunoprecipitated samples, only the upper band was detected suggesting only a protection of this high molecular form (arrowhead). Other proteins that interact with Hsp70 in an *O*-GlcNAc dependent way are in process of identification in the laboratory.

The question we asked at this stage is the relevance of such an activity for Hsp70. The hypothesis of a protection played by *O*-GlcNAc against the proteasomal degradation seems to be well accepted today. First, numerous proteins were described to be protected by the single *O*-GlcNAc glycosylation, i.e., Sp1 [4], β-estrogen receptor [5] or plakoglobin [6]; second, numerous *O*-GlcNAc sites are localized within PEST sequences usually activated by phosphorylation and we can presume that *O*-GlcNAc could protect proteins against destruction by occupying phosphorylated sites (yin–yang relationship); and third, the proteasome appears to be itself modified and regulated by *O*-GlcNAc [7,8]. Nevertheless, the role of chaperone protein in this protection process was never investigated and could appear as the "missing link." We attempt to bring a first response by searching a possible lectinic activity in

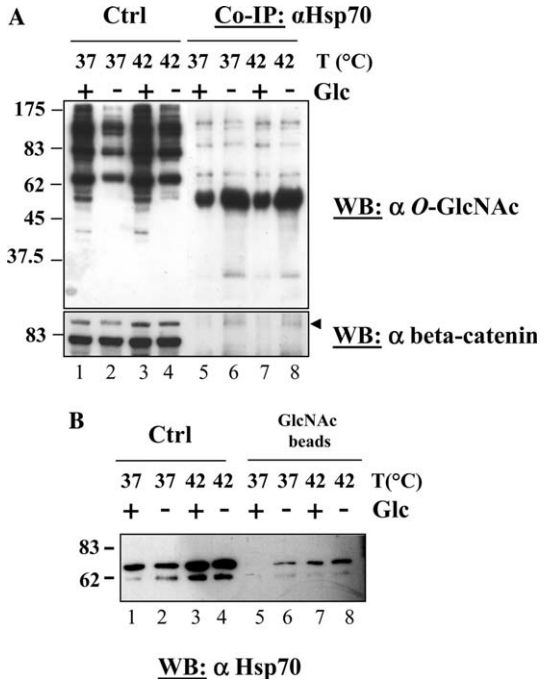

Fig. 3. Different *O*-GlcNAc proteins are associated to Hsp70, but the effect of the thermic and of the nutrient stresses did not profoundly affect their binding. HepG2 cells were cultured in the different conditions of stress (37 or 42 °C with or without glucose). Cells were lysed in a smooth lysate buffer (A, lanes 5–8) and the anti-Hsp70 antibody was added to the extract. After recovering the bound proteins with Sepharose beads-coupled protein A, proteins were run on a 10% SDS–PAGE and stained either with the anti-*O*-GlcNAc RL2 antibody as to detect the *O*-GlcNAc proteins bound to Hsp70 or with anti-β-catenin antibody. (B) The lectinic activity status of Hsp70 (as shown in Fig. 2) during the co-immunoprecipitation experiment. Ctrl, control; T, temperature in degree celsius; Co-IP, co-immunoprecipitation; and WB, Western blot.

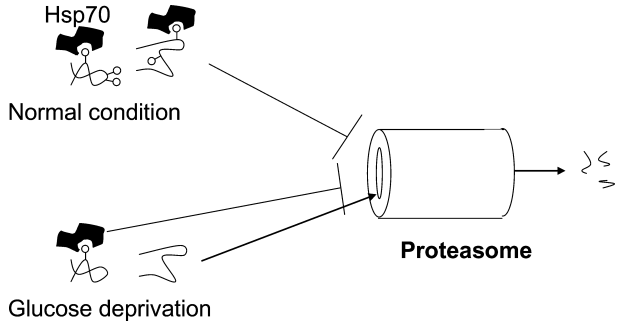

Fig. 4. Proposed mechanism of the protection of *O*-GlcNAc proteins by Hsp70 Using previous data and with the present work we have drawn this hypothesis about the protection of proteins by *O*-GlcNAc. In normal conditions (with glucose) proteins have a certain level of *O*-GlcNAc, and some of these residues are localized within PEST sequences that could be bound by HSP70 via their lectinic domain. When cells are depleted of glucose mainly the *O*-GlcNAc glycosylation decreases and only remnant *O*-GlcNAc residues are preserved, and thus permit the protein to avoid the proteasomal degradation. *O*-GlcNAc residues are represented as a white ball.

Hsp70 and to look at the modulation of this activity during stress. If *O*-GlcNAc is actually a protective signal against proteasomal degradation, chaperone proteins could play their protective role by masking PEST sequences via the glycosylation and thus permit the modified substrate to escape to the proteasomal degradation (Fig. 4).

Concluding remarks

The present work demonstrates the GlcNAc-binding activity of Hsp70 and its *O*-GlcNAc modification. Modulations of these two features in an opposite way were shown when HepG2 cells were cultured in glucose-depleted medium: the lectinic activity toward *O*-GlcNAc greatly increased in a glucose-deprived stress whereas the *O*-GlcNAc glycosylation dramatically decreased. We propose that *O*-GlcNAc proteins could be protected from proteasomal degradation by counteracting the pro-degradative effect of phosphorylation at PEST sequences by interacting with HSP70, thus reinforcing a role of *O*-GlcNAc in protein stability. This paper presents an emerging new concept in protein protection, by describing the lectinic activity of HSP70, and the new visualization of chaperone lectins by corroborating the hypothesis that *O*-GlcNAc is a glycosylation that may permit proteins to stay in life.

Acknowledgments

This work was realized in the Unité Mixte de Recherches n° 8576/CNRS (director Dr. J.-C. Michalski) of the Institut Fédératif de Recherches No.118 (director Dr. J. Mazurier). We thank Dr. Nathalie Callens for providing HepG2 cells. We are grateful to Pr. Gradimir Misevic and Dr. Yann Guerardel for critical reading of the manuscript. This work was supported in part by the CNRS and the "Génopole of Lille." Céline Guinez is a recipient of a grant from the Ministère de la Recherche et de l'Enseignement Supérieur.

References

[1] M. Rechsteiner, S.C. Rogers, PEST sequences and regulation by proteolysis, Trends Biochem. Sci. 21 (1996) 267–276.
[2] M.H. Glickman, A. Ciechanover, The ubiquitin–proteasome proteolytic pathway: destruction for the sake of construction, Physiol. Rev. 82 (2002) 373–482.
[3] S.A. Whelan, G.W. Hart, Proteomic approaches to analyze the dynamic relationships between nucleocytoplasmic protein glycosylation and phosphorylation, Circ. Res. 93 (2003) 1047–1058.
[4] I. Han, J.E. Kudlow, Reduced *O*-glycosylation of Sp1 is associated with increased proteasome susceptibility, Mol. Cell. Biol. 17 (1997) 2550–2558.
[5] X. Cheng, R.N. Cole, J. Zaia, G.W. Hart, Alternative *O*-glycosylation/*O*-phosphorylation of the murine estrogen receptor β, Biochemistry 39 (2000) 11609–11620.

[6] S. Hatsell, L. Medina, J. Merola, R. Haltiwanger, P. Cowin, Plakoglobin is O-Glycosylated close to the N-terminal destruction box, J. Biol. Chem. 278 (2003) 37745–37752.
[7] M. Sumegi, E. Hunyadi-Gulyas, K.F. Medzihradszky, A. Udvardy, 26S proteasome subunits are O-linked *N*-acetylglucosamine-modified in *Drosophila melanogaster*, Biochem. Biophys. Res. Commun. 312 (2003) 1284–1289.
[8] F. Zhang, K. Su, X. Yang, D.B. Bowe, A.J. Paterson, J.E. Kudlow, *O*-GlcNAc modification is an endogenous inhibitor of the proteasome, Cell 115 (2003) 715–725.
[9] T. Lefebvre, C. Cieniewski, J. Lemoine, Y. Guerardel, Y. Leroy, J.P. Zanetta, J.C. Michalski, Identification of *N*-acetyl-D-glucosamine-specific lectins from rat liver cytosolic and nuclear compartments as heat-shock proteins, Biochem. J. 360 (2001) 179–188.
[10] J.C. Kiang, G.C. Tsokos, Heat shock protein 70 kDa: molecular biology, biochemistry and physiology, Pharmacol. Ther. 2 (1998) 183–201.
[11] A.G. Pockley, Heat shock proteins as regulators of the immune response, Lancet 362 (2003) 469–476.
[12] F. Beaulieu, R.M. Tanguay, Members of the *Drosophila* HSP70 family share ATP-binding properties, Eur. J. Biochem. 172 (1988) 341–347.
[13] J. Imai, H. Yashiroda, M. Maruya, I. Yahara, K. Tanaka, Proteasomes and molecular chaperones: cellular machinery responsible for folding and destruction of unfolded proteins, Cell Cycle 2 (2003) 585–590.
[14] L. Wells, K. Vosseler, G.W. Hart, A role for *N*-acetylglucosamine as a nutrient sensor and mediator of insulin resistance, Cell. Mol. Life Sci. 60 (2003) 222–228.
[15] W. Zhu, B. Leber, D.W. Andrews, Cytoplasmic *O*-glycosylation prevents cell surface transport of E-cadherin during apoptosis, EMBO J. 20 (2001) 5999–6007.
[16] H. Aberle, A. Bauer, J. Stappert, A. Kispert, R. Kemler, β-catenin is a target for the ubiquitin–proteasome pathway, EMBO J. 16 (1997) 3797–3804.