CMLS, Cell. Mol. Life Sci. 60 (2003) 1217–1223
1420-682X/03/061217-07
DOI 10.1007/s00018-003-3024-9


CMLS Cellular and Molecular Life Sciences

## Research Article

# Hsp27 suppresses the formation of inclusion bodies induced by expression of R120G$\alpha$B-crystallin, a cause of desmin-related myopathy

**H. Ito*, K. Kamei, I. Iwamoto, Y. Inaguma, M. Tsuzuki, M. Kishikawa, A. Shimada, M. Hosokawa and K. Kato**

Institute for Developmental Research, Aichi Human Service Center, 713-8 Kamiya, Kasugai, Aichi 480-0392 (Japan), Fax + 81 568 88 0829, e-mail: itohide@inst-hsc.pref.aichi.jp

Received 16 January 2003; received after revision 19 February 2003; accepted 24 March 2003

**Abstract.** The R120G mutation in the small heat shock protein (sHSP) $\alpha$B-crystallin has been identified in a family suffering from desmin-related myopathy. In this study, we characterized the features of transiently expressed R120G$\alpha$B-crystallin in mammalian cells. In addition, we examined interactions of this mutant $\alpha$B-crystallin with Hsp27, another representative sHSP. In HeLa cells, transiently expressed R120G$\alpha$B-crystallin was mainly fractionated in the insoluble fraction, although wild-type $\alpha$B-crystallin was predominantly found in the soluble fraction. In immunofluorescence studies, we found 15–25% of R120G$\alpha$B-crystallin-expressing cells to contain multiple cytosolic inclusion bodies, in which Hsp27 was also localized. When R120G$\alpha$B-crystallin and Hsp27 were transiently co-expressed in HeLa cells, the amount of R120G$\alpha$B-crystallin in the soluble fraction was greater than with expression of R120G$\alpha$B-crystallin alone. Moreover, co-expression resulted in reduced formation of inclusion bodies, suggesting that Hsp27 acts as a molecular chaperone for R120G$\alpha$B-crystallin.

**Key words.** Crystallin; Hsp27; chaperone; inclusion body; myopathy.

Molecular chaperones participate in folding of many proteins under both unstressed and stressed conditions [1]. Several families are known to exist in mammalian cells, including the small heat shock protein (sHSP) family, which have a molecular mass of 15–30 kDa and share several features: possession of a homologous amino acid sequence called the '$\alpha$-crystallin domain' [2]; induction by various stimuli including heat, oxidative reagents and heavy metals [3, 4], and phosphorylation concomitant with dissociation from large oligomers in response to various stresses and growth factors [5–8]. $\alpha$B-crystallin is a sHSP known to be abundant in unstressed mammalian tissues such as ocular lens, cardiac and skeletal muscles [9]. There are several reports describing accumulation of $\alpha$B-crystallin in the brains of patients suffering from degenerative ailments such as Alexander's [10] and Alzheimer's disease [11], although the physiological significance is not clear. Recently, a missense mutation in $\alpha$B-crystallin, R120G, was found in a French family suffering from desmin-related myopathy (DRM) [12], an autosomal dominant myopathy characterized by cytoplasmic aggregates of desmin in muscle cells [13]. There have been several reports concerning the nature of R120G$\alpha$B-crystallin in vitro and in whole animals. Using a recombinant protein purified from bacterial culture, the R120G mutation has been shown to alter the structure and chaperone-like activity of $\alpha$B-crystallin [14–16]. Another group produced transgenic mice expressing R120G$\alpha$B-crystallin and reported that they exhibit symptoms similar to DRM [17].

Hsp27 is another representative member of the sHSP family, also abundant in muscle cells [18]. Together with

* Corresponding author.

αB-crystallin, it forms large heterooligomers in mammalian cells and tissues [19, 20], although the relationship between the two proteins in mammalian cells has not been elucidated. In the present study, we therefore characterized the nature of R120GαB-crystallin in mammalian cell lines transiently expressing the mutant protein using biochemical and cell-biological methods. In addition, we examined interactions with constitutively and exogenously expressed Hsp27.

## Materials and methods

### Reagents

Okadaic acid and calyculin A were purchased from Wako Pure Chemicals (Osaka, Japan) and the protease inhibitor cocktail and mouse monoclonal antibodies against vimentin (clone V9) and β-tubulin (clone TUB 2.1) from Sigma (St Louis, Mo.). Alexa Fluor 488 goat anti-rabbit IgG and Alexa Fluor 568 goat anti-mouse IgG conjugates were obtained from Molecular Probes (Eugene, Ore.). Fluorescein isothiocyanate-labeled goat anti-mouse IgG and rhodamine-labeled goat anti-rabbit IgG were purchased from Bio Source International (Camarillo, Calif.). The green fluorescent protein (GFP) expression vector, pEGFP-C3, was obtained from BD Biosciences Clontech (Palo Alto, Calif.).

### Construction of expression vectors

For expression of αB-crystallin in mammalian cells, a *Hin*dIII and *Not*I fragment from human αB-crystallin cDNA (generously provided by Dr. A. Iwaki, Kyushu University, Japan) [21] was inserted into the expression vector pRc/CMV (Invitrogen, Tokyo, Japan). For site-directed mutagenesis, we used the polymerase chain reaction (PCR) with oligonucleotide mutation primers and a template αB-crystallin expression vector. *Hin*dIII and *Not*I fragments from PCR fragments were inserted into *Hin*dIII and *Not*I sites of pRc/CMV. We constructed two plasmids to express wild-type αB-crystallin and αB-crystallin in which Arg120 was substituted with glycine, and designated these plasmids as wtαB-pRc/CMV and R120GαB-pRc/CMV. The mutated sites were confirmed by DNA sequencing. An *Eco*RI fragment from the human Hsp27 cDNA cloned from a cDNA library of HeLa cells was inserted into the expression vector pCMV5 (generously provided by Dr. H. Itoh, Nara Institute of Science and Technology, Japan) [22].

### Cell culture and preparation of cell extracts

HeLa and CHO cells were grown in Dulbecco's modified Eagle's medium (DMEM; Nissui Pharmaceutical, Tokyo, Japan), supplemented with 10% fetal calf serum (Equitech-Bio, Ingram, Tex.). Cells were seeded in 35-mm dishes, transfected with 1 μg of plasmids using Lipofectamine PLUS reagent (Invitrogen), harvested 24–48 h alter, and suspended in 200 μl of 50 mM Tris-HCl buffer, pH 7.5, containing 0.1 M NaF, 5 mM EDTA, 0.2 μM okadaic acid, 0.2 μM calyculin A and 1/100 volume of the protease inhibitor cocktail. We designated this buffer as buffer S. Each suspension was sonicated at 0 °C and centrifuged at 125,000 g for 20 min at 4 °C. The supernatants were used as the soluble fractions. The pellets were washed once by sonication and centrifugation with buffer S and then solubilized with 100 μl of buffer S containing 2% sodium dodecyl sulfate (SDS). The resultant supernatants were used as the insoluble fractions.

### Electrophoresis and Western blot analysis

SDS-polyacrylamide gel electrophoresis (PAGE) was performed as described by Laemmli [23] with 12.5% gels. The included proteins were transferred electrophoretically to nitrocellulose sheets, incubated at room temperature for 2 h with primary antibodies, and then for 1 h with secondary antibodies. Affinity-purified antibodies raised in rabbits against human Hsp27 [19] and the carboxyl-terminal decapeptide of αB-crystallin [9] were employed as the first, and peroxidase-labeled antibodies raised in goat against rabbit IgG as the second antibodies. For detection of GFP, a mouse monoclonal antibody against GFP (clone 1E4; Medical & Biological Laboratories, Nagoya, Japan) and peroxidase-labeled antibodies raised in goat against mouse IgG (Medical & Biological Laboratories) were used. Peroxidase activity on membranes was visualized on X-ray films using a Western blot chemiluminescence reagent (Western Lightning; Perkin Elmer Life Sciences, Boston, Mass.).

### Immunofluorescence

Cells were seeded on glass coverslips and transfected with various plasmids. After 24–48 h, each coverslip was rinsed twice with phosphate-buffered saline (PBS; containing 8 g NaCl, 0.2 g KCl, 1.15 g $Na_2HPO_4 \cdot 12\ H_2O$ and 0.2 g $KH_2PO_4$ in 1000 ml $H_2O$), treated with PBS containing 0.5% Nonidet P-40 (NP-40) and 5 mM $MgCl_2$ for 1 min at room temperature, washed three times with PBS and fixed with 4% paraformaldehyde in PBS for 30 min at room temperature. In some experiments, cells treated with various chemicals were washed with PBS, fixed with 4% paraformaldehyde and treated with 0.2% Triton X-100 in PBS for 15 min at room temperature. Fixed cells were washed three times with PBS and blocked with 10% normal goat serum in PBS containing 0.1% sodium azide for 30 min. Incubation was with primary and secondary antibodies, diluted in 10% normal goat serum in PBS, at room temperature for 2 and 1 h, respectively. Fluorescent images were obtained using an Axiovert 200 fluorescent microscope with an attached AxioCam HRc digital camera (Carl Zeiss, Oberkochen, Germany) and a FLUOVIEW confocal microscope (Olympus Optical, Tokyo, Japan).

**Immunoelectron microscopy**

HeLa cells were fixed with 4% paraformaldhyde in PBS and then washed with 0.1 M phosphate buffer, pH 7.4 containing 4.5% sucrose. After washing, the specimens were dehydrated in graded ethanol and embedded in a mixture of Epon (TAAB Laboratories, Aldermaston, UK) and Araldite (Nisshin EM, Tokyo, Japan). Ultrathin sections (70 nm thick) were collected over nickel grids using an 8800 Ultramicrotome III (LKB, Bromma, Sweden). Immunogold labeling was carried out by a post-embedding method using affinity-purified rabbit anti-αB-crystallin (1 µg/ml) followed by biotinylated goat antibody to rabbit IgG (1:200 dilution; Vector Laboratories, Burlingame, Calif.) and a streptavidin colloidal gold conjugate (20 nm diameter, 1:10 dilution; EY Laboratories, San Mateo, Calif.). Immunostained sections were post-fixed with 1% glutaraldehyde in PBS and counterstained with 2% uranyl acetate and 1% lead citrate. Images were obtained using a JEOL 1200EX II electron microscope (Tokyo, Japan).

**Evaluation of the inclusion body-containing cells**

Cells were stained for immunofluorescence as described above. The number of transfected cells with and without inclusion bodies were counted in five randomly chosen microscope fields in different areas of a coverslip. Data are expressed as percentage of total transfected cells with inclusion bodies. To examine the effects of co-expression of Hsp27 and GFP, cells were transfected with 0.5 µg of R120GαB-pRc/CMV and various amounts of Hsp27/pCMV5 or EGFP-C3 and expression of the heat shock proteins and GFP were determined by Western blot analysis.

**Other methods**

Human Hsp27 was purified from samples of pectoral muscles obtained at surgical resection of breast cancer [19]. Bovine αB-crystallin was purified from lens as described previously [9]. Concentrations of protein were estimated using a micro BCA protein assay reagent kit (Pierce, Rockford, Ill.) with bovine serum albumin as the standard.

## Results

**Cellular distribution of R120GαB-crystallin in transiently transfected HeLa cells**

As shown in figure 1, transiently expressed wtαB-crystallin was mainly fractionated in the soluble fraction, whereas R120GαB-crystallin was predominantly found in the insoluble fraction, the total contents of both proteins in transiently transfected HeLa cells being almost equal. By immunofluorescence microscopy, wtαB-crystallin was observed mainly in the cytosol after treatment of cells with a detergent after fixation (fig. 2A). HeLa cells constitutively expressed a significant amount of Hsp27, another representative small heat shock protein, in the cytosol and this localization did not appear to be affected by expression of wtαB-crystallin (fig. 2A). R120GαB-crystallin was also observed in the cytosol in many cells although some contained multiple inclusion body-like structures that were intensely stained (fig. 2A; indicated with arrows) or were stained only peripherally (fig. 2A; indicated with arrowheads) with antibodies against αB-crystallin. These structures were first seen after 8 h of transfection (data not shown). We observed inclusion body-like structures when we transfected with 0.2–1 µg of R120GαB-crystallin-expressing plasmids and the number of these structure-containing cells increased in a dose-dependent manner (data not shown). When we transfected 1 µg of R120GαB-crystallin-expressing plasmids and estimated the ratio of these structure-containing cells after 24 h of transfection, inclusion body-like structures were observed in 15–25% of cells that were transfected with R120GαB-crystallin, although the percentage varied between experiments. Formation of inclusion body-like structures after transfection with R120GαB-crystallin was also observed in CHO and COS-m6 cells (data not shown), and thus was not specific to HeLa cells. Hsp27 was also observed in the inclusions (fig. 2A), and in the cells with this feature, the staining of Hsp27 appeared to be relatively weak compared to cells lacking these structures (fig. 2A). Since R120GαB-crystallin was distributed in the insoluble fraction (fig. 1), we treated cells with a detergent before fixation to wash out the soluble components and then stained with individual antibodies. Immunostaining of Hsp27 and αB-crystallin in cells transfected with wtαB-crystallin almost completely disappeared (fig. 2B). In contrast, intense staining of inclusion body-like structures was observed in some cells transfected with R120GαB-crystallin. Hsp27 was also localized in these structures (fig. 2B). The removal by detergent before fixation of unknown soluble compo-

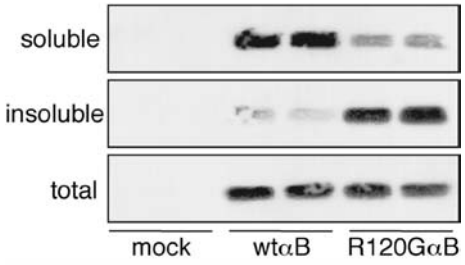


Figure 1. Expression of wtαB-crystallin and R120GαB-crystallin in HeLa cells. HeLa cells were transfected with plasmids to express wtαB-crystallin (wtαB) or R120GαB-crystallin (R120GαB) and soluble, insoluble and whole-cell extracts containing 5 µg of proteins were subjected to SDS-PAGE and subsequent Western blot analysis using antibodies against the carboxyl-terminal peptide of αB-crystallin. 'mock' represents cells transfected with empty vector.

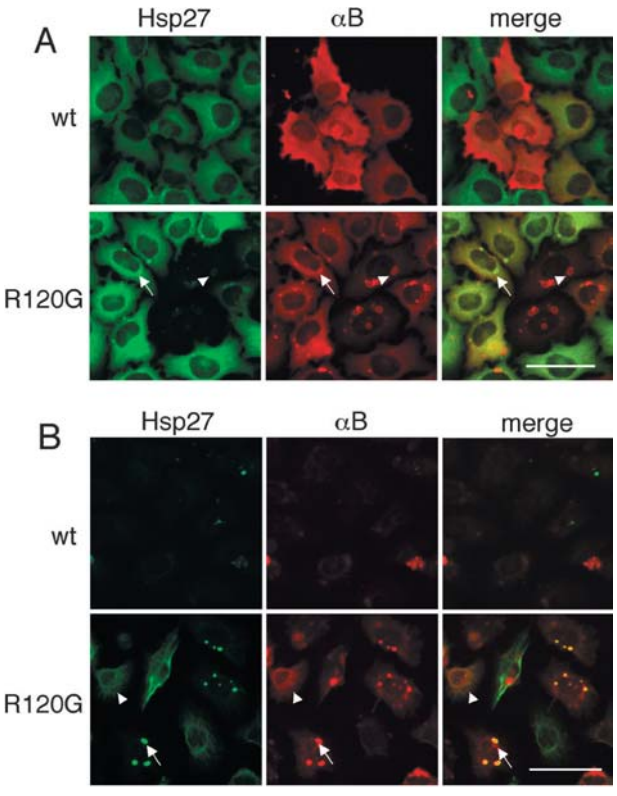


Figure 2. Localization of wtαB-crystallin, R120GαB-crystallin and Hsp27 in HeLa cells transfected with wtαB-crystallin or R120GαB-crystallin. Cells were transfected with wtαB-crystallin (wt) or R120GαB-crystallin (R120G) and incubated at 37 °C for 24 h. After washing, cells were fixed and then treated with a detergent (*A*) or with a detergent before fixation (*B*) and subsequently stained with antibodies against Hsp27 or αB-crystallin (αB). All images were obtained using a fluorescence microscope with an attached digital camera. Bars, 50 µm. (*A*) Inclusion bodies that were well stained (arrow) or stained only peripherally (arrowhead) are indicated. (*B*) An inclusion body (arrow) and a filamentous structure (arrowhead) are indicated.

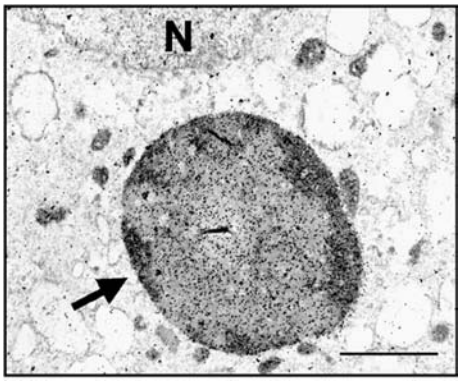


Figure 3. Immunoelectron micrograph of HeLa cells expressing R120GαB-crystallin. Immunoelectron microscopy using antibodies against the carboxyl terminal of αB-crystallin was performed as described in Materials and methods. N, nucleus. The arrow indicates an inclusion body containing R120GαB-crystallin. Bar, 1 µm.

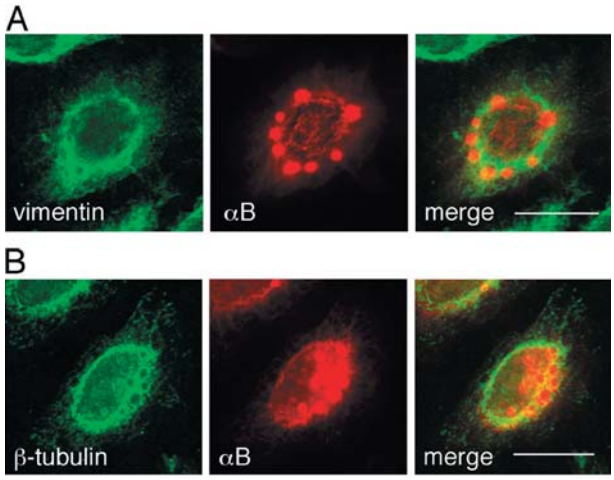


Figure 4. Localization of vimentin (*A*) and β-tubulin (*B*) in HeLa cells transiently expressing R120GαB-crystallin. HeLa cells were transfected to express R120GαB-crystallin. After 24 h, they were washed, treated with a detergent, fixed and stained with antibodies against αB-crystallin and against vimentin (*A*) or β-tubulin (*B*). Bar, 20 µm.

nent(s) that interfere with antibody binding was presumably responsible for these differences in inclusion body staining. On immunoelectron microscopic analysis, as shown in figure 3, intense signals for αB-crystallin were observed in the inclusions, confirming that the expression of R120GαB-crystallin caused the formation of inclusion bodies in HeLa cells. Along with the staining of αB-crystallin in inclusion bodies in cells expressing R120GαB-crystallin, filamentous staining was observed (fig. 2B). Because αB-crystallin is known to interact with tubulin and intermediate filaments such as vimentin, we compared the staining of R120GαB-crystallin with those of β-tubulin and vimentin. The filamentous staining of R120GαB-crystallin differed from that of vimentin (fig. 4A) and β-tubulin (fig. 4B). Moreover, vimentin and β-tubulin were not accumulated in the inclusion bodies (fig. 4).

### Effects of co-expression of Hsp27 on the solubility of R120GαB-crystallin and formation of inclusion bodies

αB-crystallin and Hsp27 are known to interact and form heterooligomers in mammalian cells and tissues [19, 20]. We therefore examined whether co-expression of Hsp27 affects the solubility of R120GαB-crystallin in mammalian cells. When 0.5 µg of R120GαB-crystallin-expressing plasmid was transfected with various amounts of Hsp27-expressing plasmid, solubilization of R120GαB-crystallin was observed even at the ratio of 1:0.1 (fig. 5A). To determine whether this was a specific effect of Hsp27, we performed a co-expression experiment with R120GαB-crystallin and GFP. As shown in figure 5B, the

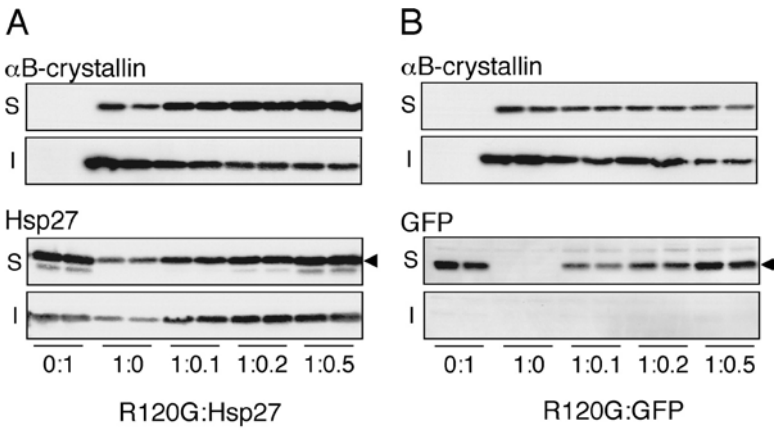


Figure 5. Co-expression of Hsp27 changes the distribution of R120G$\alpha$B-crystallin in HeLa cells. Cells were transfected with the indicated ratios of R120G$\alpha$B-crystallin and Hsp27 (*A*) or GFP (*B*). After 24 h, they were harvested and soluble (S) and insoluble (I) extracts were obtained, and extracts containing 5 µg of protein were subjected to SDS-PAGE with subsequent Western blot analysis using antibodies against $\alpha$B-crystallin (upper panels) and against Hsp27 or GFP (lower panels).

solubilization of R120G$\alpha$B-crystallin was not observed at any ratios although the levels of R120G$\alpha$B-crystallin in both soluble and insoluble fractions tended to decrease at higher ratios. Co-expression of Hsp27 at a ratio of 1 : 0.1 markedly reduced the formation of inclusion bodies due to expression of R120G$\alpha$B-crystallin, complete inhibition being evident at a ratio of 1 : 0.2 (table 1). Expression of GFP at a ratio of 1 : 0.1 did not reduce inclusion body-containing cells although the expression of GFP at higher ratios (1 : 0.2 to 1 : 1) tended to decrease inclusion body-containing cells (table 1).

Table 1. Co-expression of Hsp27 suppresses the formation of inclusion bodies induced by R120GαB-crystallin in HeLa cells.

| Transfection | Transfected cells with inclusion bodies (%) |
|---|---|
| R120G$\alpha$B-crystallin alone | 21.1 ± 2.23 |
| R120G$\alpha$B-crystallin : Hsp27 | |
| 1 : 0.1 | 2.97 ± 0.540 |
| 1 : 0.2 | 0.00 ± 0.00 |
| R120G$\alpha$B-crystallin : GFP | |
| 1 : 0.1 | 19.8 ± 1.22 |
| 1 : 0.2 | 12.0 ± 1.46 |

Transfected cells were counted under fluorescence microscopy to calculate the percentage containing inclusion bodies. Each value is the mean ± SD of results from three samples.

## Discussion

The present study showed R120G$\alpha$B-crystallin to predominantly locate in insoluble components of cells, including cytosolic inclusion bodies (figs. 1–3). R120G$\alpha$B-crystallin has been reported to cause the formation of aggregates including desmin, a member of the type III intermediate filament family, in C2.7 myoblasts and BHK21 kidney smooth muscle cells [12], but morphological features are quite different from the structures in HeLa cells, which did not contain a significant amount of desmin (fig. 2). HeLa cells contain vimentin, another type III intermediate filament member, but this was not a feature of inclusion bodies induced by expression of R120G$\alpha$B-crystallin (fig. 4 A). Since Perng et al. [16] reported an increased binding ability of R120G$\alpha$B-crystallin to glial fibrillary acidic protein (GFAP) filaments compared with wt$\alpha$B-crystallin in vitro, these results suggest that the mutant protein may preferentially induce aggregation of desmin and GFAP, but not vimentin.

Expression of misfolded proteins such as a mutant of the cystic fibrosis transmembrane conductance regulator (CFTR), and treatment with a proteasome inhibitor may create the cytoplasmic inclusion body called the aggresome [24, 25]. The aggresome is now defined as a pericentriolar, membrane-free, cytoplasmic inclusion containing misfolded, ubiquitinated proteins ensheathed in a cage of intermediate filaments, whose formation is inhibited by microtubule-disrupting agents such as nocodazole [24, 25]. Recently, we reported that proteasome inhibitors induce the accumulation, phosphorylation and recruitment of Hsp27 and $\alpha$B-crystallin into aggresomes in U373 MG human glioma cells [26]. The inclusion bodies induced by expression of R120G$\alpha$B-crystallin in this study, however, did not contain the intermediate filament,

vimentin (fig. 4A). Moreover, they were negative for ubiquitin, and nocodazole did not inhibit their formation (data not shown), indicating a nature distinct from the previously reported aggresomes.

Hsp27 and $\alpha$B-crystallin are known to form hetero-oligomers that have an apparent molecular mass of about 500 kDa in unstressed mammalian cells and tissues [19, 20]. In this study, we examined the effect of Hsp27 on the solubility of R120G$\alpha$B-crystallin and the formation of inclusion bodies. Co-expression of Hsp27 with R120G$\alpha$B-crystallin caused a remarkable solubilization of R120G$\alpha$B-crystallin without a significant increase in Hsp27 in the insoluble fraction (fig. 5). In addition, formation of inclusion bodies by expression of R120G$\alpha$B-crystallin was also inhibited by the co-expression of Hsp27 (table 1), indicating that Hsp27 is a powerful chaperone for the mutant protein.

Co-expression of GFP with R120G$\alpha$B-crystallin did not cause solubilization of R120G$\alpha$B-crystallin although the levels of R120G$\alpha$B-crystallin in both soluble and insoluble fractions and the number of cells with inclusion bodies tended to decrease at higher ratios of co-transfection (fig. 5, table 1). The reasons for these phenomena are unclear. We think that co-expression of GFP with R120G$\alpha$B-crystallin may be rather toxic for cells, the expression of R120G$\alpha$B-crystallin may be decreased and as a result, the number of inclusion body-containing cells was reduced.

There have been reports that insolubilization of proteins and formation of inclusion bodies may cause cell death [27]. However, cells with inclusion bodies induced by R120G$\alpha$B-crystallin in the present study did not exhibit typical morphological features of cell death such as shrinkage and nuclear fragmentation (fig. 2 and data not shown). Moreover, culturing for up to 96 h after transfection did not result in increased loss of cells (data not shown). Since lens and muscle tissues seem to be selectively affected by the R120G$\alpha$B-crystallin mutation [12], the toxic effects of R120G$\alpha$B-crystallin may be restricted to these tissues and thus we could not detect a visible toxicity toward the epithelial HeLa cell line.

In conclusion, R120G$\alpha$B-crystallin exists in insoluble protein complexes and causes the formation of inclusion bodies in mammalian cells. Moreover, co-expression of Hsp27 may result in solubilization and reduction of such inclusion bodies, pointing to a possible therapeutic application in patients suffering from DRM due to $\alpha$B-crystallin mutation.

*Acknowledgements.* This work was supported in part by Grants-in-Aid for Scientific Research and the Priority Areas (C)-Advanced Brain Science Project from the Ministry of Education, Science, Sports, Culture and Technology of Japan.

1 Hartl F. U. (1996) Molecular chaperones in cellular protein folding. Nature **381:** 571–579
2 Caspers G. J., Leunissen J. A. and Jong W. W. de (1995) The expanding small heat-shock protein family, and structure predictions of the conserved '$\alpha$-crystallin domain'. J. Mol. Evol. **40:** 238–248
3 Klemenz R., Fröhli E., Steiger R. H., Schäfer R. and Aoyama A. (1991) $\alpha$B-crystallin is a small heat shock protein. Proc. Natl. Acad. Sci. USA **88:** 3652–3656
4 Kato K., Goto S., Hasegawa K. and Inaguma Y. (1993) Coinduction of two low-molecular-weight stress proteins, $\alpha$B crystallin and HSP28, by heat or arsenite stress in human glioma cells. J. Biochem. (Tokyo) **114:** 640–647
5 Arrigo A.-P. (1990) Tumor necrosis factor induces the rapid phosphorylation of the mammalian heat shock protein hsp28. Mol. Cell. Biol. **10:** 1276–1280
6 Landry J., Chretinen P., Laszlo A. and Lambert H. (1991) Phosphorylation of HSP27 during development and decay of thermotolerance in Chinese hamster cells. J. Cell. Physiol. **147:** 93–101
7 Kato K., Hasegawa K., Goto S. and Inaguma Y. (1994) Dissociation as a result of phosphorylation of an aggregated form of the small stress protein, hsp27. J. Biol. Chem. **269:** 11274–11278
8 Ito H., Kamei K., Iwamoto I., Inaguma Y., Nohara D. and Kato K. (2001) Phosphorylation-induced change of the oligomerization state of $\alpha$B-crystallin. J. Biol. Chem. **276:** 5346–5352
9 Kato K., Shinohara H., Kurobe N., Inaguma Y., Shimizu K. and Ohshima K. (1991) Tissue distribution and developmental profiles of immunoreactive $\alpha$B crystallin in the rat determined with a sensitive immunoassay system. Biochim. Biophys. Acta. **1074:** 201–208
10 Iwaki T., Kume-Iwaki A., Liem R. K. H. and Goldman J. E. (1989) $\alpha$B crystallin is expressed in non-lenticular tissues and accumulates in Alexander's disease brain. Cell **57:** 71–78
11 Shinohara H., Inaguma Y., Goto S., Inagaki T. and Kato K. (1993) $\alpha$B crystallin and HSP28 are enhanced in the cerebral cortex of patients with Alzheimer's disease. J. Neurol. Sci. **119:** 203–208
12 Vicart P., Caron A., Guicheney P., Li Z., Prévost M. C., Faure A. et al. (1998) A missense mutation in the $\alpha$B-crystallin chaperone gene causes a desmin-related myopathy. Nat. Genet. **20:** 92–95
13 Goebel H. H. (1995) Desmin-related neuromuscular disorders. Muscle Nerve **18:** 1306–1320
14 Kumar L. V., Ramakrishna T. and Rao C. M. (1999) Structural and functional consequences of the mutation of a conserved arginine residue in $\alpha$A and $\alpha$B crystallins. J. Biol. Chem. **274:** 24137–24141
15 Bova M. P., Yaron O., Huang Q., Ding L., Haley D. A., Stewart P. L. et al. (1999) Mutation R120G in $\alpha$B-crystallin, which is linked to a desmin-related myopathy, results in an irregular structure and defective chaperone-like function. Proc. Natl. Acad. Sci. USA **96:** 6137–6142
16 Perng M. D., Muchowski P. J., Den I. P. van, Wu G. J., Hutcheson A. M., Clark J. I. et al. (1999) The cardiomyopathy and lens cataract mutation in $\alpha$B-crystallin alters its protein structure, chaperone activity, and interaction with intermediate filaments in vitro. J. Biol. Chem. **274:** 33235–33243
17 Wang X., Osinska H., Klevitsky R., Gerdes A. M., Nieman M., Lorenz J. et al. (2001) Expression of R120G-$\alpha$B-crystallin causes aberrant desmin and $\alpha$B-crystallin aggregation and cardiomyopathy in mice. Circ. Res. **89:** 84–91
18 Inaguma Y., Hasegawa K., Goto S., Ito H. and Kato K. (1995) Induction of the synthesis of hsp27 and $\alpha$B crystallin in tissues of heat-stressed rats and its suppression by ethanol or $\alpha$1-adrenergic antagonist. J. Biochem. (Tokyo) **117:** 1238–1243
19 Kato K., Shinohara H., Goto S., Inaguma Y., Morishita R. and Asano T. (1992) Copurification of small heat-shock protein

with αB-crystallin from human skeletal muscle. J. Biol. Chem. **267:** 7718–7725
20 Kato K., Goto S., Hasegawa K., Shinohara H. and Inaguma Y. (1993) Responses to heat shock of αB crystallin and HSP28 in U373 MG human glioma cells. Biochim. Biophys. Acta. **1175:** 257–262
21 Iwaki A., Iwaki T., Goldman J. E., Ogomori K., Tateishi J. and Sakaki Y. (1992) Accumulation of αB-crystallin in brains of patients with Alexander's disease is not due to an abnormality of the 5′-flanking and coding sequence of the genomic DNA. Neurosci. Lett. **140:** 89–92
22 Andersson S., Davis D. L., Dahlback H., Jornvall H. and Russell D. W. (1989) Cloning, structure, and expression of the mitochondrial cytochrome P-450 sterol 26-hydroxylase, a bile acid biosynthetic enzyme. J. Biol. Chem. **264:** 8222–8229
23 Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685
24 Johnston J. A., Ward C. L. and Kopito R. R. (1998) Aggresomes: a cellular response to misfolded proteins. J. Cell Biol. **143:** 1883–1898
25 García-Mata R., Bebok Z., Sorscher E. J. and Sztul E. S. (1999) Characterization and dynamics of aggresome formation by a cytosolic GFP- chimera. J. Cell Biol. **146:** 1239–1254
26 Ito H., Kamei K., Iwamoto I., Inaguma Y., García-Mata R., Sztul E. et al. (2002) Inhibition of proteasomes induces accumulation, phosphorylation, and recruitment of HSP27 and αB-crystallin to aggresomes. J. Biochem. (Tokyo) **131:** 593–603
27 Sherman M. Y. and Goldberg A. L. (2001) Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. Neuron **29:** 15–32

.