Relocalization of αB-crystallin by heat shock in ovarian carcinoma cells

Christina E.M. Voorter, Liesbeth Wintjes, Hans Bloemendal and Wilfried W. de Jong

Department of Biochemistry, Centre of Eye Research, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

αB-Crystallin, a major lens protein, is present in clearly detectable amounts in cultured ovarian carcinoma cells. After heat-shock treatment of these cells at 45°C αB-crystallin relocalizes from the detergent-soluble, cytosolic fraction to the non-ionic detergent-insoluble nuclear/cytoskeletal fraction. Colchicine treatment of the cells, although giving rise to a vimentin collapse on the nucleus, does not result in redistribution of αB-cyrstallin. When this colchicine treatment is followed by heat shock, αB-crystallin relocalizes again to the insoluble fraction, indicating that this relocalization is independent of the collapse of the vimentin network.

Small heat-shock protein; Stress; Non-lenticular tissue; Eye lens; Intermediate filament

1. INTRODUCTION

α-Crystallin, one of the most abundant proteins in vertebrate eye lenses, occurs as large aggregates (of up to 10^6 Da), composed of two types of related subunits, αA and αB, and a variety of post-translational modifications thereof [1]. Both subunits show a striking sequence homology with the sHSPs (15–30 kDa), most notably in their C-terminal halves [2,3]. Like α-crystallin, these sHSPs occur as large aggregates (200–800 kDa) forming spherical structures 15–20 nm in diameter [1,4,5]. Recently it has been shown that considerable amounts of αB-crystallin are also present in many non-lenticular tissues, notably in heart, skeletal muscles and kidney [6–8]. Furthermore, accumulation of αB-crystallin was observed in brains of patients with certain degenerative neurological disorders [9–13] and in NIH 3T3 mouse fibroblasts expressing Ha-*ras* and v-*mos* oncogenes [14], which implicates a relation with cellular stress conditions. Considering these findings it is suggested that the resemblance between α-crystallin and sHSPs is not restricted to structural features alone, but might be extended to functional properties as well, although the biochemical functions of the sHSPs are still largely unknown. Indeed, the synthesis of αB-crystallin can be induced by stress treatments like heat, arsenite [15] and osmotic shock [16], in agreement with the presence of a heat-shock consensus element in the promoter region of the gene for αB-crystallin [17]. Also, the fact that both sHSPs and αB-crystallin can be phosphorylated [18,19] suggests a functional similarity between these two proteins.

One of the characteristics of the sHSPs is their association with the nucleus under heat-shock conditions, followed by a slow relocalization to the cytoplasm [5,20]. In this article we describe a comparable phenomenon for αB-crystallin in ovarian carcinoma cells, and we demonstrate that the relocalization of αB-crystallin from detergent-soluble to the insoluble fraction under heat-shock conditions is not simply due to an entrapment by the vimentin filaments, which collapse onto the nucleus under these conditions.

2. MATERIALS AND METHODS

2.1. *Cell culture and stress treatments*

Human ovarian carcinoma cells, designated COV413B (C.A.M. van den Berg-Bakker, unpublished data), were kindly provided by Dr. Peter Schrier, Department of Clinical Oncology, University Hospital, Leiden, The Netherlands. This cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and maintained in a humidified atmosphere of air enriched with 5% CO_2 at 37°C unless indicated otherwise. Cells were treated with colchicine at a concentration of 1 μg/ml at 37°C for 3.5 h, or were heat shocked by incubation at 45°C for 3.5 h, or both treatments were performed successively. After the different treatments cells were either used for immunoblotting or for immunofluorescence analysis.

2.2. *Immunoblotting*

After the different treatments 413B cells from a T75 flask were washed twice with PBS and scraped from the flask with a rubber policeman in PBS. Cells were pelleted at 500 × *g* for 5 min and cell fractionation was performed by non-ionic detergent lysis using a buffer containing 10 mM Tris (pH 7.5), 5 mM $MgCl_2$, 10 mM NaCl, 0.1 mM phenylmethylsulfonylfluoride and 0.5% (v/v) Triton X-100. Detergent-soluble and insoluble fractions were separated by centrifugation in an Eppendorf centrifuge for 5 min, and the insoluble fractions were washed once with the same buffer. Fractions were dissolved in SDS sample buffer and proteins were separated by one-dimensional SDS-gel electrophoresis according to Laemmli [21]. Immunoblot analysis was performed essentially as described by Mulders et al. [22]. The

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primary antiserum was raised in rabbits against bovine lens αB-crys-
tallin and used at a dilution of 1:200. Unfortunately, this antiserum
gave no satisfactory results in immunofluorescence studies on cells.

2.3. Immunofluorescence

After the appropriate incubations 413B cells growing on glass cover
slips were washed twice with PBS and fixed in methanol at −20°C for
10 min, dipped in cold acetone and air-dried. Aspecific reactions were
blocked by a 15 min pre-incubation in normal goat serum diluted 1:10
in PBS. Anti-vimentin serum used for staining was diluted 1:20 in PBS.
whereas goat anti-rabbit antibody coupled with fluorescein isothiocy-
anate at a dilution of 1:10 in PBS was used as a second antibody. All
incubations were performed for 30 min at room temperature followed
by extensive washing with PBS. Cells were examined and photo-
graphed with an Olympus BH2 fluorescence microscope.

3. RESULTS

In our investigation of the presence of αB-cyrstallin
in cells under pathological conditions we found detecta-
ble amounts of this protein in an ovarian carcinoma cell
line, called 413B (see Fig. 1, lane 1). We used this cell
line to investigate the temperature-dependent intracellu-
lar localization of αB-crystallin. Cells grown in flasks
for 3 days were heated at 45°C or treated with colchi-
cine for 3.5 h, or both treatments were performed suc-
cessively. Using non-ionic detergent lysis and centrifu-
gation, a separation could be obtained between a cy-
tosolic and a crude nuclear/cytoskeletal fraction. In
each case equal portions of the supernatant and pellet
were applied to the gel and the relative distribution of
αB- crystallin was determined by Western blot analysis.
Fig. 1 reveals that αB-crystallin does indeed occur in the
same fraction as the non-nuclear proteins at physiolog-
ical temperatures (lanes 1,2) and mainly with crude nu-
clei after heat treatment (lanes 5,6). In contrast, neither
treatment of the cells with colchicine, a microtubuli poi-
son, nor arsenite at a concentration of 75 μM (data not
shown) resulted in a relocalization of αB-crystallin from
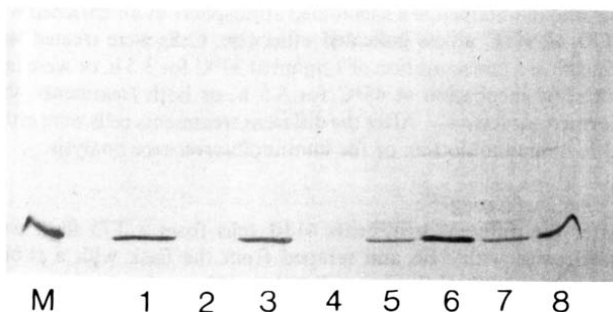the cytosolic to the nuclear/cytoskeletal fraction (Fig. 1,

Fig. 1. Redistribution of αB-crystallin under stress conditions. 413B
cells were incubated for 3.5 h at 37°C (lanes 1,2), at 37°C in the
presence of colchicine (lanes 3,4), at 45°C (lanes 5,6), and treated with
colchicine followed by a heat shock at 45°C (lanes 7,8). Cells were
detergent lysed, and soluble (lanes 1,3,5,7) and insoluble (lanes 2,4,6,8)
fractions were analyzed by Western blot, using an antibody against
bovine αB-crystallin. Samples from equal amounts of cells were loaded
on each gel track. Purified bovine lens αB-crystallin was used as a
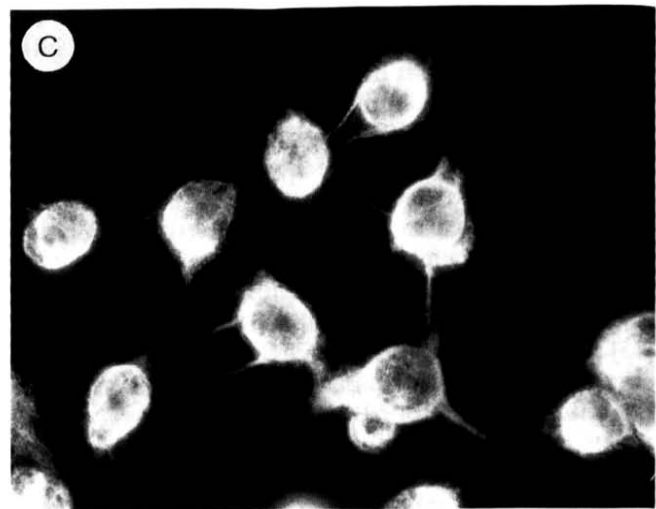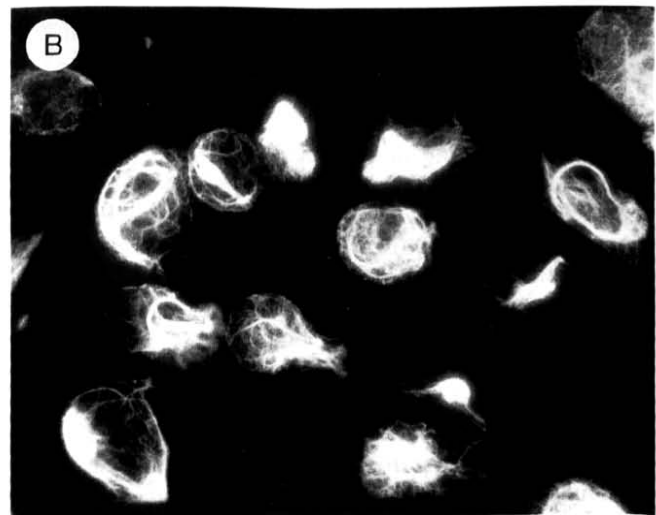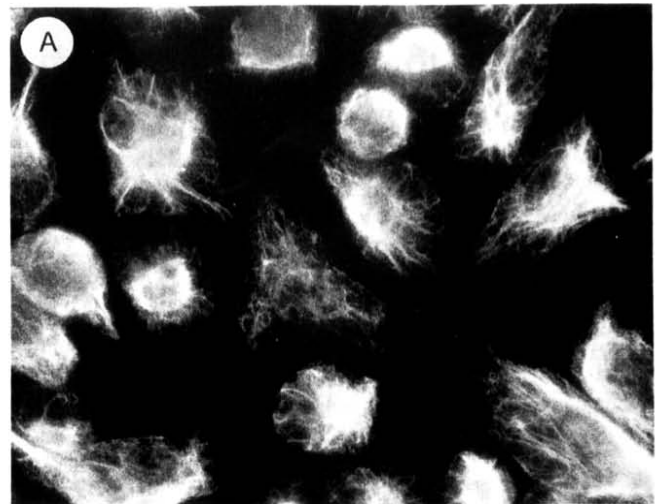marker (lane M).

Fig. 2. Localization of vimentin in 413B cells under stress conditions
by indirect immunofluorescence. Cells grown on glass cover slips were
incubated for 3.5 h at 37°C in the absence (A) or presence (B) of
colchicine or at 45°C (C). Cells were stained with an antibody against
vimentin and visualized with FITC-conjugated goat anti-rabbit IgG
antibodies.

lanes 3,4), even though arsenite is known as an inducer of the stress response.

Both colchicine and arsenite treatments, as well as heating at 45°C, resulted in a redistribution of the vimentin-containing intermediate filaments into a tight network around the nucleus in the majority of these ovarian carcinoma cells, as can be seen from Fig. 2. The treatment with colchicine resulted in a ribbon-like pattern of intermediate filaments, which was morphologically distinct from the heat-shock induced collapse of the intermediate filament structure (cf. Fig. 2B and C). Such a difference in intermediate filament structure by different stress conditions has also been observed in chicken embryo fibroblasts [20]. The fact that the conditions for redistribution of αB-crystallin are not concordant with those of vimentin already suggests that both processes occur independently. This was further examined by analyzing the distribution of αB-crystallin in detergent-lysed cells first exposed to colchicine, which promotes the collapse of vimentin, and subsequently given a heat-shock treatment. As can be seen in Fig. 1, lanes 7 and 8, much of the αB-crystallin again redistributed into the insoluble fraction of these cells.

4. DISCUSSION

The biochemical data presented here show that αB-crystallin in ovarian carcinoma cells undergoes dynamic intracellular reorganization in response to the stress state of the cell. A similar phenomenon has been observed in rat astrocytoma cells [23] and NIH 3T3 cells [15]. Comparable relocalizations from the detergent-soluble to the insoluble nuclear/cytoskeletal fraction were found for the sHSPs in chicken and rat fibroblasts [19,20], HeLa cells [24] and Drosophila S3 cells [25]. However, the vimentin-containing intermediate filaments also undergo redistribution from their normal cytoplasmic localization into a meshwork of filaments wrapped around the nucleus shortly after heat shock (for review see [26]). Therefore, it has been suggested that the relocalization of the sHSPs is a consequence of this collapsed intermediate filament network, either due to an association of these proteins with the cytoskeleton or simply by an entrapment in a non-specific fashion within the collapsing intermediate filaments. Although immunofluorescence data support an association of the sHSPs with the intermediate filament cytoskeleton in Drosophila Kc cells [27], Arrigo and co-workers [5] found that in HeLa cells the redistribution of HSP28 into the detergent-insoluble nuclear/cytoskeletal fraction after heat shock is not simply a consequence of the intermediate filament collapse.

α-Crystallin has also been suggested to associate with the cytoskeleton [28], and therefore a co-collapse of the vimentin-skeleton and αB-crystallin under heat-shock conditions would be possible. However, in this study we clearly show that the colchicine-induced collapse of in-

termediate filaments and the redistribution of αB-crystallin are independent events.

The presence of αB-crystallin in the non-ionic detergent-insoluble fraction after heat shock may result from an actual localization of this protein in the nucleus or in distinct insoluble aggregates in the cytoplasm. For the sHSPs both possibilities have been described; chicken fibroblasts HSP24 and Drosophila HSP23 in cultured cells appear as clusters of aggregates in an equatorial perinuclear ring upon various stress treatments [20,29], whereas Drosophila HSP23 in salivary glands and mammalian HSP28 in HeLa cells are localized in the nucleus after heat shock [5,30]. Concerning αB-crystallin, the formation of insoluble aggregates at elevated temperatures, although not impossible, is not a property of the protein per se since purified αB-crystallin failed to form non-ionic detergent-insoluble aggregates upon comparable heat-shock conditions (unpublished data).

In the case of the sHSPs both the significance of this intracellular distribution as well as the overall functional aspects of these proteins remain unclear. Some putative role for HSP27 as a protective molecule during thermal stress has been assessed [31] and a positive correlation was found between the amount of HSP27 expressed and survival of Chinese hamster cells after treatment with various anticancer drugs [32]. Comparable suggestions have been made for αB-crystallin, which is considered to be a true member of the class of sHSPs [15]. However, until now, the biological function(s) of αB-crystallin, both in the eye lens as well as in other tissues, remains to be elucidated.

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