Translocation of β crystallin in neural cells in response to stress

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Abstract While extralenticular expression of proteins in the α crystallin (small heat shock protein) family is well documented, that for proteins in the β/γ-superfamily is less well established. Here we show, using SDS-PAGE, Western blotting and confocal microscopy, that there is a constitutive level of β crystallin expression in mouse N1E-115 neural cells. Furthermore, upon heat shock at 43°C or 55°C, or cold shock at 30°C, β crystallin immunoreactivity translocated predominantly from the nuclear region into the cytoplasmic region of the cells. In conditions of stress, it may be important for β crystallin to be recruited into the cytoplasm to stabilise other proteins via its high β-sheet content, and/or to ensure that storage levels of cytoplasmic Ca^{2+} are maintained.

Key words: Greek key; Immunoglobulin; Lens; Blindness; Neuroblastoma

1. Introduction

The eye lens contains high concentrations of soluble proteins, the crystallins. They fall into two classes, the α crystallin family and the β/γ-superfamily [1]. There is differential expression of the crystallins during lens development [2] which leads to different mixtures of crystallins along the visual axis. Properties of individual crystallins may be important in maintaining short range order, and thus transparency, in the lens [3]. Many of the crystallins are expressed extralenticularly; for example the mammalian small heat shock protein α crystallin is emerging as a key protein in a remarkable variety of cellular processes including: oxidative stress responses in heart and respiratory tissue [4], development of receptivity in the secretory phase endometrium [5], cellular differentiation in the eye, and in a variety of neurodegenerative disorders. We have shown that heat shock of a N1E-115 neural cell line results in translocation of over-expressed αB crystallin from the cytoplasm to the nuclear region, and that mutations in αB crystallin that effect its chaperone-like activity also influence viability of N1E-115 neural cells under stress, while not influencing the distribution of the protein within the cell [6,7].

By contrast, the case for extralenticular expression and function of β crystallin is less well established. βB2 and βA3/A1 crystallins have been found in the chick retina [8], and βB2 crystallin in the mammalian retina [9]. In this paper we show, using immunocytochemistry and confocal microscopy, that β crystallin is expressed endogenously in N1E-115 neural cells, and that temperature-induced stress results in translocation of the protein predominantly into the cytoplasm. This has important implications for the role of the protein in cells under stress.

2. Materials and methods

2.1. Cell culture

N1E-115 cells, from a mouse neuroblastoma cell line, were obtained from ECACC (ECACC No. 88112303) and grown from frozen stock by the following protocol: 5 ml of Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% foetal calf serum, 2 mM glutamine, 200 U/ml penicillin and 100 μg/ml streptomycin (referred to later as 10% DMEM) was placed in a culture flask and cell stock solution was added. The flask was mixed gently and placed horizontally for 30–60 min to allow viable cells to adhere. The medium was replaced with fresh medium and the flask incubated at 37°C, 5% CO_2.

For routine passaging, the medium was removed from a 25 ml flask and the cell sheet washed with 5 ml phosphate buffered saline (PBS), 1 mM EDTA. The cell sheet was covered with 1 ml PBS/EDTA/Trypsin (the latter at 0.5 g/l) and incubated for 10–15 min at 37°C. To 1 ml of cell suspension, 4 ml of the medium described above was added and this suspension was further diluted into a flask containing fresh medium for onward growth.

2.2. Gel electrophoresis

SDS polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting using anti-β_H crystallin antibody (which also reacts with β_L crystallin) was performed as described previously [6,10].

2.3. Temperature shock

Heat shock experiments were carried out in 25 ml flasks or slide flasks (Life Technologies), as appropriate. Cells were seeded in the flasks in 5 ml or 3 ml respectively of 10% DMEM supplemented with selection antibiotics as required and allowed to grow to 50–60% confluency. Cells were shocked by incubation of the flasks at 30°C, 34°C, 43°C, or 55°C in 5% CO_2 for 1 h, 3 h or 6 h as required.

2.4. Confocal microscopy

Cell sheets on the slide surface were washed with PBS and then fixed with freshly prepared 4% paraformaldehyde for 10 min at room temperature. Blocking was done with 1% foetal calf serum in PBS, 0.05% azide for 1 h at room temperature. Following four washes of the cells with PBS, cells were permeabilised by incubation with 50% methanol, 50% acetone for 10–15 min at room temperature. Cells were washed four times with PBS prior to use of the primary antibody. This rabbit anti-rat β_H crystallin polyclonal antibody serum [10] was used at a 1:1000 dilution in PBS and incubation was for 1 h at room temperature. The fluorescence antibody (goat anti-rabbit IgG-FITC conjugate, Vector Laboratories, UK) was added at 1:500 dilution in PBS and incubation followed for 1 h at room temperature in the dark. After a final four washes with PBS, the cells were mounted in Vectorlabs H-1300 mounting medium containing propidium iodide, covered by a coverslip (0.17 ± 0.1 μm) and stored in the dark until examined by confocal microscopy, using a Leica DMRBE microscope with TCSNT software.

3. Results

Western blotting of proteins from the stable N1E-115 cell line with anti-β_H crystallin antibody showed only a single reactive band, which migrated in the position expected for

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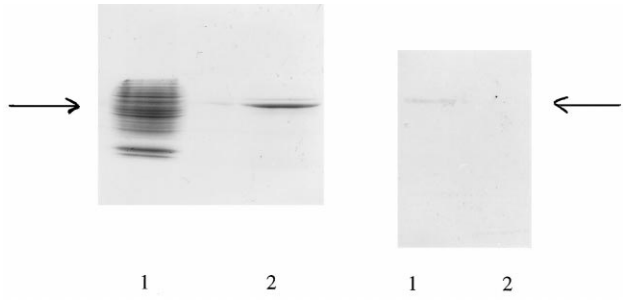

Fig. 1. β Crystallin identification in N1E-115 mouse neuroblastoma cells. Left panel: SDS polyacrylamide gel electrophoresis; lanes are as follows: 1, N1E-115 cells; 2, medium after incubation and separation of cells by centrifugation. The arrow shows the migration position of β crystallin. Right panel: Western blot; lanes are as follows: 1, N1E-115 cells; 2, medium after incubation and separation of cells by centrifugation. The arrow shows the migration position of β crystallin.

β_H crystallin (Fig. 1). No β crystallin was found in the medium after extraction of the cells. Temperature-induced stress did not appear to result in significantly increased immunoreactivity, therefore expression, of β crystallin.

Confocal microscopy showed that immunofluorescence for β crystallin in unstressed N1E-115 cells was located predominantly in the nuclear region of the cells (Fig. 2B). The control without antibody showed no immunofluorescence (Fig. 2A). Heat stress at 43°C for 3 h resulted in movement of the immunoreactivity to the cytoplasmic area of the cells (Fig. 2C); results were identical for 6 h heat stress, and for cold shock at 30°C for 3 or 6 h. More severe heat stress at 55°C for 3 or 6 h showed almost complete translocation of the immunoreactivity to the cytoplasm (Fig. 2D).

4. Discussion

SDS-PAGE, Western blotting and confocal microscopy show that there is a constitutive level of β crystallin expression in mouse N1E-115 neural cells. This is of interest, because it is generally assumed that evolution of the β/γ-superfamily has involved specialisation for the lens environment.

Upon temperature-induced stress, β crystallin immunoreactivity translocated predominantly from the nuclear region into the cytoplasmic region of the cells. This is in contrast to the movement of αB crystallin in N1E-115 cells and in NIH 3T3 cells upon heat shock, which is in the opposite direction, from the cytoplasm to the nucleus, to interact with intermediate filament proteins or with DNA [7,11]; but in accord with the movement of αB crystallin on hypertonic shock in N1E-115 cells with high KCl ([7], Wiessman, Coop, Goode and Crabbe, unpublished results).

Paired β-sheets such as those seen in the Greek key/Immunoglobulin fold are employed in a wide variety of proteins to facilitate protein-protein interactions [12,13]. The simplest and earliest members of immunoglobulin family functioned as

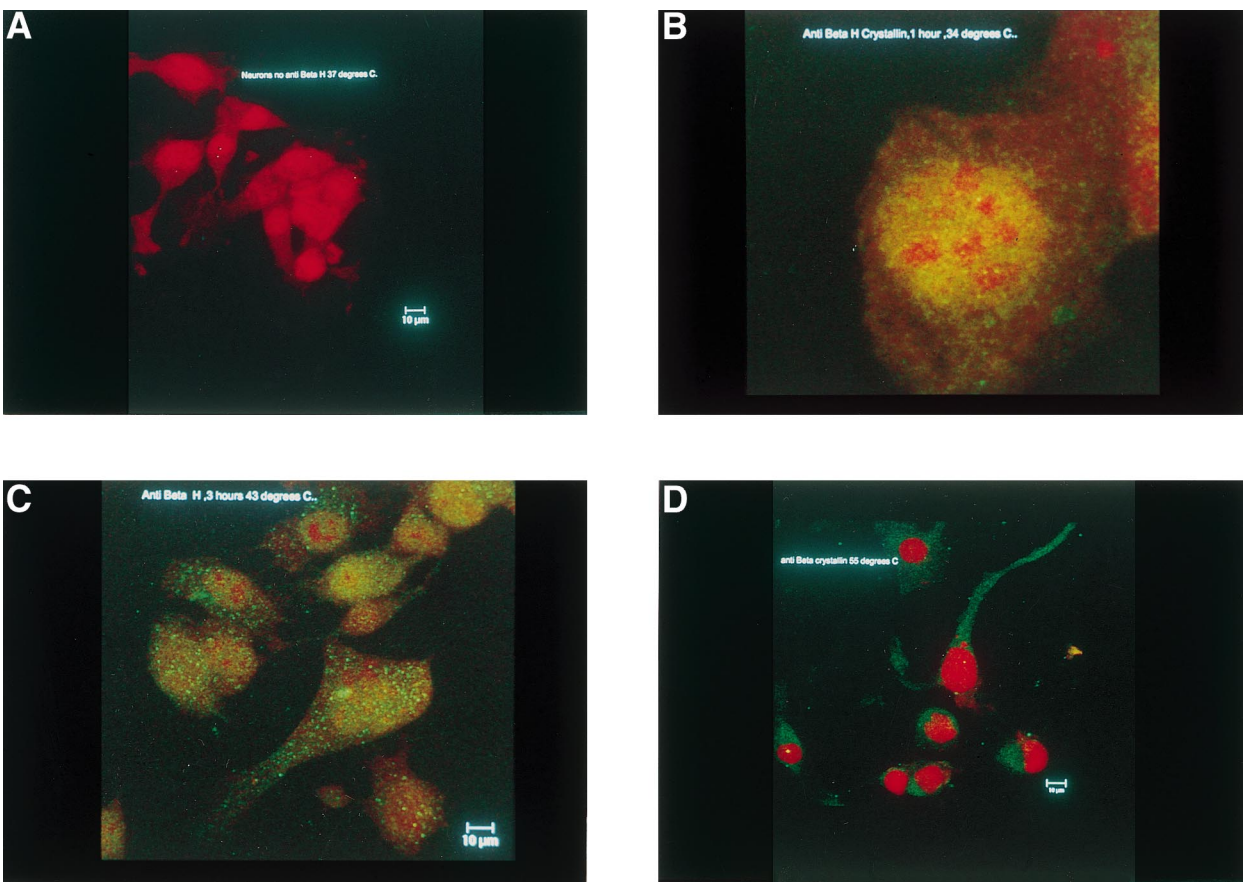

Fig. 2. Confocal microscopy of N1E-115 mouse neuroblastoma cells. A: Cells without anti-β_H crystallin antibody; B: cells with anti-β_H crystallin antibody, 1 h at 34°C; C: cells with anti-β_H crystallin antibody, 3 h at 43°C; D: cells with anti-β_H crystallin antibody, 3 h at 55°C.

simple cellular adhesives [14]. The thermal stability and Ca^{2+}-binding capacity [15] of β crystallin are both useful properties under conditions of cellular stress. It has been suggested that stress-related properties such as these represent a common feature, and may indicate the mechanism by which proto-crystallin proteins were selected or recruited for high-level expression in the lens [16]. In conditions of stress in neural cells, it may be important for β crystallin to be recruited into the cytoplasm to stabilise other proteins via its high β-sheet content, and/or to ensure that storage levels of cytoplasmic Ca^{2+} are maintained.

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