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Research Article

IN-VITRO INTERACTION OF αB-CRYSTALLIN ON SERUM AMYLOID A AND SERUM AMYLOID A FIBRILS WITH BC3H1 CELLS

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ABSTRACT

The BC3H1 smooth muscle cells of mice brain, BC3H1 is a permanent cell line derived from a mouse brain tumor. When these cells become quiescent, they express the muscle form of creatine phosphokinase, myokinase, the nicotinic acetylcholine receptor, as well as smooth muscle a-actin, all indicative of a muscle phenotype. This is important in relation to the activity of membrane proteins, because losing the activity of such systems will ultimately lead to malfunction or death of the cell. The interactions of Serum Amyloid A (SAA) and Serum Amyloid A protofibrils with BC3H1 cells of the mouse are dealt with in detail to study the binding of SAA protofibrils in various onditions. The induced fluorescence, induced circular dichroism, FACScan and MTT assay results have shown the SAA and SAA prototfibrils binding and cell toxicity with the BC3H1 cells with different concentrations of alphaB-Crystallin 0.15-15 nM. Specifically, cells were incubated with 1.25-6.25 μM SAA-FITC and SAA protofibrils-FITC assayed. The 50% viable BC3H1 cells at 4–6 μM with an LD_{50} of 3.5 μM. The interaction of serum amyloid A fibrils with a cell surface binding site/receptor might alter the local environment to cause cellular dysfunction and to be more favorable for amyloid formation. In the present study, concluding that the SAA fibrils and SAA protein binding and cell cytotoxicity was reduced in the presence of alphaB-Crystallin.

INTRODUCTION

Small heat-shock proteins (sHSPs) are one of four families of heat-shock proteins (HSPs) expressed in response to heat shock and other forms of stress (Parsell and Lindquist 1993). They are a diverse family of proteins that appear to be ubiquitous in nature, being found as surface antigens in eukaryotic parasites, as inclusion body-binding protein in E. Coli, and as structural proteins in the vertebrate lens (Caspers *et al.,* 1995). Despite their low molecular mass (12-40 kDa), sHSPs are isolated as large oligomeric complexes of 2-40 subunits depending on the physiological state of the cell. Distant members of the family have relatively low sequence similarity except for a highly conserved stretch of 100 amino acids, often called the α-Cyrstallin domain (Caspers *et al.,* 1995). Although their cellular function under physiological conditions is still largely unknown, the increased expression of sHSP is associated with cell survival under heat stress (Arrigo and Landry 1994). The role of these proteins in thermotolerance appears to be a consequence of their ability to function as molecular chaperones and to modulate actin filament dynamics (Lee *et al.,* 1995).

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sHSPs from many species inhibit the unfolding-induced aggregation of proteins in an ATP-independent manner and form a stable complex with their protein substrate. Thus, during periods of stress, sHSPs act as energy-independent traps preventing the irreversible aggregation of proteins. Recently, it has been shown that upon establishment of refolding conditions, proteins bound to sHSPs are efficiently refolded in cooperation with other chaperones (Lee *et al.,* 1997)

The evaluation of the role of the α-Crystallin domain and consequent insight into the molecular mechanisms involved in these diverse functions are hampered by the lack of structural information on HSP 27 and sHSPs in general. The major difficulty relates to the considerable flexibility of their quaternary structure (Groenen *et al.,* 1994). This flexibility, believed to be critical for their function, results in conformational heterogeneity that to date has prevented the crystallization of these proteins. Therefore, there is limited information concerning the tertiary structure of the subunits and their arrangement in the oligomer. It has been proposed that mammalian sHSPs have a tertiary structure similar to that proposed for lens α-Crystallin subunits (De Jong *et al.,.,* 1993). The model, proposed by Wistow, consists of two domains, each of two structurally similar motifs with a dynamic C-terminal arm (Wistow 1993).

On the secondary structure level, HSP 27 appears to be composed mainly of β-sheets as revealed by far-UV CD (Merck *et al.,* 1993). Electron microscopy studies indicate that mouse HSP25 forms ring-like particles, Behlke *et al.* (1991) proposed that these particles are composed of 32 monomers in hexagonal packing. The research subunit is investigating the in-vivo functions of the lens α-Crystallin proteins (αA and αB).

These proteins, abundant in vertebrate lenses, were originally thought to be solely structural proteins, but in recent years have been shown to possess a myriad of activity in-vitro including molecular chaperone activity, autokinase activity, DNA binding activity and binding to and regulating the Polymerization State of several cytoskeletal proteins. Mice lacking the α-Crystallins have been generated and are being analyzed. Mice lacking αB-Crystallin appear to be completely normal. Mice lacking αA however develop cataracts beginning early in life and progressing in severity with age. In the absence of αA, the αB forms large insoluble masses, 1 to 3 um in diameter, in the cytoplasm in lens fiber cells. Lenses of mice lacking αB-Crystallin appear to be completely normal. However, these mice exhibit dystrophy of select skeletal muscles, particularly those of the head (especially tongue) and those surrounding the spine. Mice lacking both α-Crystallins exhibit a severe cataract very early in life and also develop the muscular dystrophy observed in αB-Crystallin knockout mice (Xu *et al.,* 2000).

The BC3H1 cell line is a nonfusing muscle cell line that has been particularly useful in this regard. These cells do not fuse, but under appropriate conditions, differentiate. Differentiation is defined here as a large increase in the rate of synthesis of proteins characteristic of mature muscle such as M-CPK, vascular smooth muscle a-actin (a-actin), and the nicotinic acetylcholine receptor (AChR) (Glaser and Wice 1989). Recently demonstrated that the acidic form of brain-derived fibroblast growth factor as well as impure commercial pituitary-derived fibroblast growth factor will advance quiescent BC3H1 cells from Go 4-6 h into the GI portion of the cell cycle and at the same time repress the synthesis of the muscle form of creatine phosphokinase (Wice *et al.,* 2987). Number of investigations has demonstrated the usefulness of causing quiescent, differentiated myoblasts under nonfusing conditions to reenter the cell cycle in response to a mitogenic stimulation. A cell line particularly suitable for these studies is the muscle-like cell line, BC3H1 (Lathrop *et al.,* 1985). The role of αB-Crystallin in the development of these diseases is largely unknown but may be related to its functional role as a small heat shock protein (Caspers *et al.,* 1995). Recently, α-Crystallin was reported to act as a chaperone molecule capable of protecting other proteins from denaturation (Horwitz, 1992; Horwitz 1993). Whether this chaperone function is active in-vivo either in the lens, or, in other tissues, is unknown. To understand the effect of αB-Crystallin on Aβ fibril formation, we need to determine whether they interact with each other. In the present study, in-vitro studies on the role of the interaction of SAA/SAA fibrils with chaperones were investigated. The results indicated that there was interaction between SAA and αB-Crystallin when they were incubated together with BC3H1 Cells.

A possible mechanism for this interaction and its implicated significance in-vitro are discussed.

MATERIALS AND METHODS

Isolation of αB-Crystallin

Bovine lenses (8-10 lenses) were decapsulated and homogenized (Luthra and Balasubramanian 1993) in 0.1 M Tris buffer, pH 7.4 containing 0.5 M NaCl, 1 mM EDTA, and 0.1% NaN_3. The insoluble protein fraction and membrane debris was removed by centrifugation at 30,000g for 30 min. The supernatant was chromatographed on a Sephacryl-S200 (Pharmacia Biotech, Uppsala, Sweden) gel filtration column (0.8 × 90 cm) to separate the alphaB-Crystallin using Gradifrac™ FPLC system (Pharmacia Biotech, Uppsala, Sweden). Each of the alphaB-Crystallin fractions was dialyzed repeatedly against water using FILTRON concentrating 10000 D. Molecular weight cut off membrane (Gelman Sciences, India) and lyophilized and stored at -20°C. Protein was determined by Lowry *et al.,* (Lowry *et al.,* 1951). Homogeneous 12% SDS-polyacrylamide gels were run on the mini gel electrophoresis system (Laemmli, 1970).

Isolation of SAA

SAA was isolated from the plasma of casein injected (Botto *et al.,* 1997) mice as reported previously by Lindhorst *et al.,* (Lindhorst *et al.,* 1997). It was then characterized using 17% SDS-polyacrylamide gels. Isolated SAA was purified by Waters reverse phase-high performance liquid chromatography (Kaplan *et al.,* 1999) (RP-HPLC) analytical column 4.6 × 250 mm Spherisorb ODS2 LC_{18} (Waters, Milford, Massachusetts, USA) and size exclusion chromatography (SEC) columns using a series of 7.8 × 300 mm Ultrahydrogel 250™ and Ultrahydrogel 500™ the molecular weight was determined (Waters, Milford, Massachusetts, USA).

Fluorolabelling of SAA and SAA fibrils

Purified SAA and SAA proptofibrils (400 μg/400 μl 0.001% NH_4OH, pH 9.0) were labeled with fluorecein isothiocyanate (FITC) (Fluka, USA) by dialysis through a 1000 D cutoff membrane. FITC (0.4 mg) was dissolved in 40 μl DMSO and added to 40 ml of PBS adjusted to pH 9.0 with NH_4OH. After 12 hrs at 4°C the SAA-FITC and SAA protofibrils-FITC were dialyzed against milli-Q water, sterile filtered (0.45 μm) and held at 4°C (Linke *et al.,* 1991). 1.25-6.25 μM of SAA-FITC and SAA protofibrils-FITC were assayed with 0.15-15 nM of α-Crystallin.

Single Photon Counting Life-time Measurements

1.25-6.25 μM of SAA-FITC and SAA protofibrils-FITC was assayed with 0.15-15 nM of αB-Crystallin for lifetime measurements (FL, at λ_{max} 530±15 nm) (Spectra physics, IBH Consultants, Scotland, UK).

Induced Circular Dichroic Spectroscopic Studies

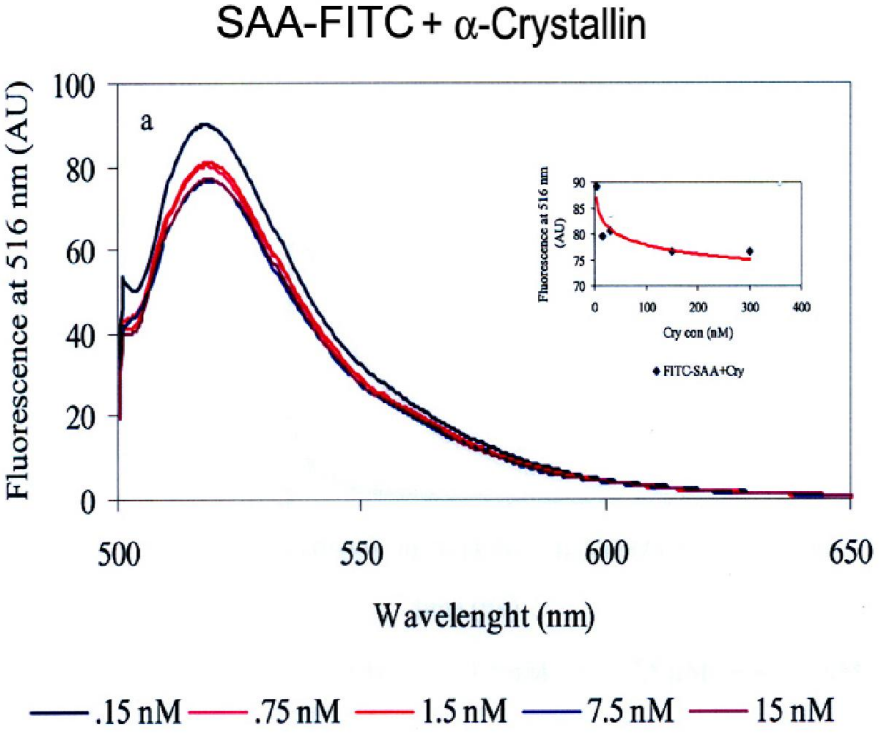
1.25-6.25 μM of SAA-FITC and SAA protofibrils-FITC were assayed with 0.15-15 nM of αB-Crystallin for induced CD measurements (Jasco J-715 Spectropolarimeter, Tokyo, Japan).

FACScan Analysis: BC3H1 Cells.

Mice BC3H1 cells were incubated with 1.25-6.25 µM of SAA-FITC and SAA protofibrils-FITC assayed with 0.15-15 nM of αB-Crystallin for 24 hrs at 4°C, washed in PBS, fixed immediately in 1% paraformaldehyde in PBS, and assayed for cellular fluorescence (FL, at λ_{max} 530±15 nm), forward scatter (FSC), and side scatter (SSC). Binding was analyzed on a FACScan flow cytometer using FACScan analysis software WINMDI (Becton Dickinson, San Jose, CA, USA).

MTT assay

1.25-6.25 µM of SAA-FITC and SAA protofibrils-FITC were assayed with 0.15-15 nM of αB-Crystallin (Rousset 2001) for incubation with BC3H1 cells for 24 hrs. BC3H1 cells were fed with 200 µL fresh medium at the ends of the growth period and added 50 µl of MTT to all wells in columns 1 to 6. Wrapped plates in aluminum foil and incubated for 4 hrs in a humidified atmosphere at 37°C. This is a minimum incubation time and plates can be left for up to 8 hrs.



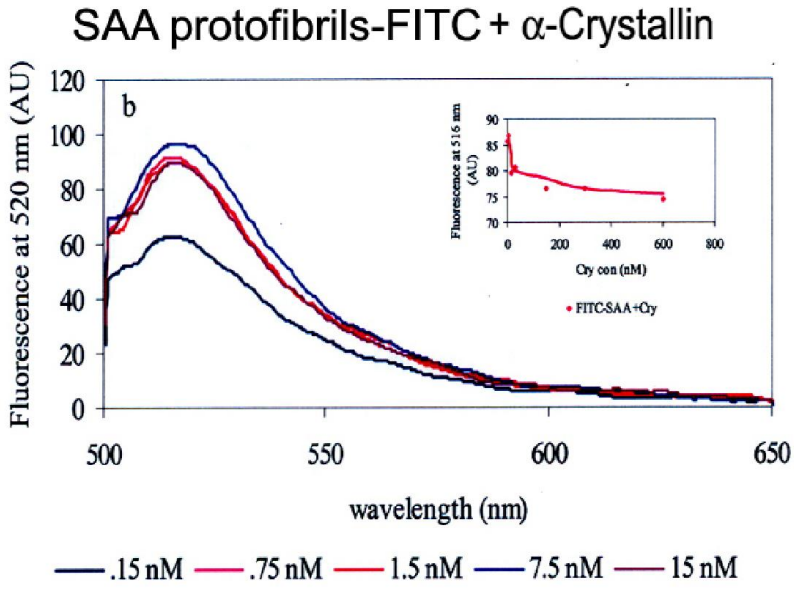


Fig. 1. Fluorescence spectra of SAA-FITC (a) SAA-FITC (2.5 mM) with increasing concentration of (0.15-15 nM) alphaB-Crystallin and insert plot shows concentration Vs Fluorescence, (b) SAA-FITC protofibrils (2.5 mM) with increasing concentration (0.15-15 nM) alphaB-Crystallin and insert plot shows concentration Vs Fluorescence

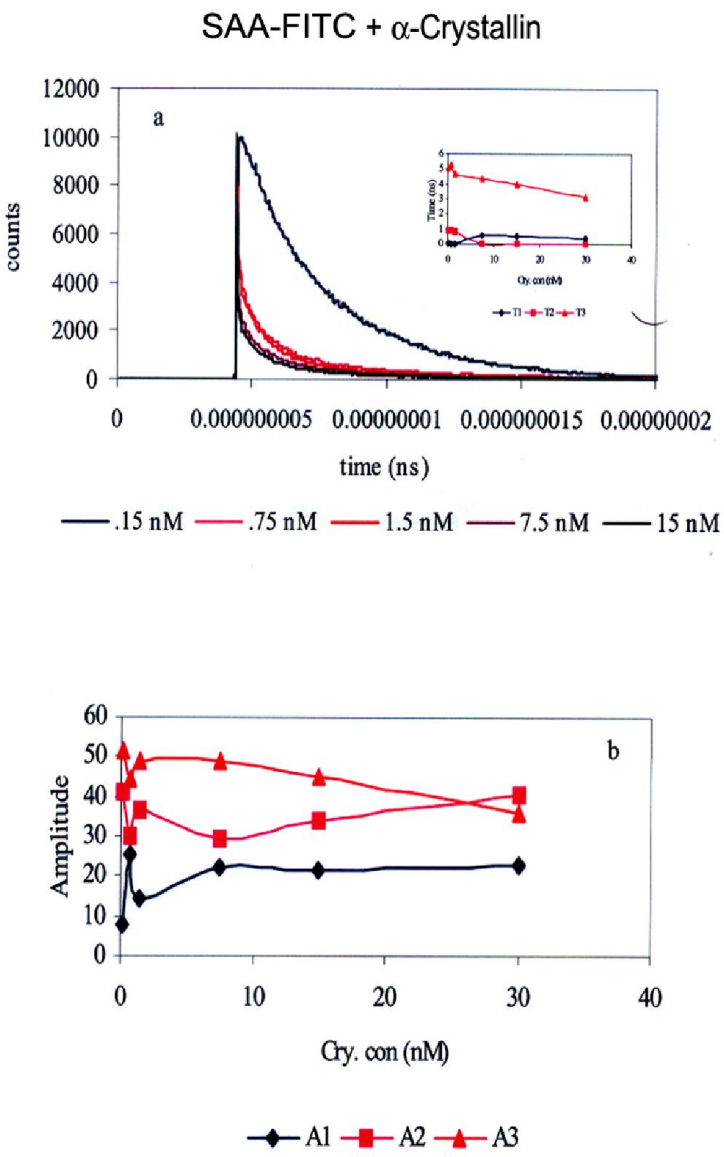


Fig. 2. Life time measurement fluorescence decay of SAA-FITC (a) SAA-FITC (2.5 mM) with increasing concentration of (0.15-15 nM) alphaB-Crystallin and insert plot shows concentration Vs time, (b) plot shows concentration Vs amplitude

Removed the medium and MTT from the wells and dissolved the remaining MTT-formazan crystals, adding 200 µl of DMSO to all wells in columns 1 to 6. Added glycine buffer (25 µl per well) to all wells containing the DMSO. Recorded absorbance at 570 nm immediately, since the product was unstable. The wells in column 1, which contained medium, MTT, but no cells, were used to blank the plate reader.

RESULTS

The fluorescence spectral changes observed on αB-Crystallin binding to FITC-SAA was shown in spectrum Fig. 1a. During addition of αB-Crystallin, the fluorescence at 516 nm decreased gradually indicating that FITC-fluorescence was quenched by the disulfide bonds in αB-Crystallin. The fluorescence intensity was increased during addition of αB-Crystallin to SAA protofibril-FITC. This increase in FITC intensity may be associated with the conversion of protofibrils by αB-Crystallin in lower concentration (at 5 nM).

However, further addition of αB-Crystallin does not change the fluorescence intensity. The effect of αB-Crystallin concentration on lifetime of FITC-SAA protofibril is indicated in Fig. 1b. The average fluorescence lifetime decreased from 3.0 to 1.2 ns indicating that the flourophore is in highly polar environment than the previous state. In high αB-Crystallin concentration (30 nM), there was quenching of FITC in SAA by disulfide groups around the environment. The amplitude for long lifetime (τ_1) was decreased significantly from 50% to 40% during addition of αB-Crystallin. However, short-lived species was constant during further αB-Crystallin incubation (Fig. 2a and b).

From the induced CD studied, changes in FITC environment of SAA were measured at different concentrations of αB-Crystallin. The CD band at 450 and 505 nm were changed during addition of αB-Crystallin. The measured ellipticity values were plotted against various concentrations of αB-Crystallin which, is given in Fig. 3a. The ellipticity attained saturation at 2 nM αB-Crystallin.

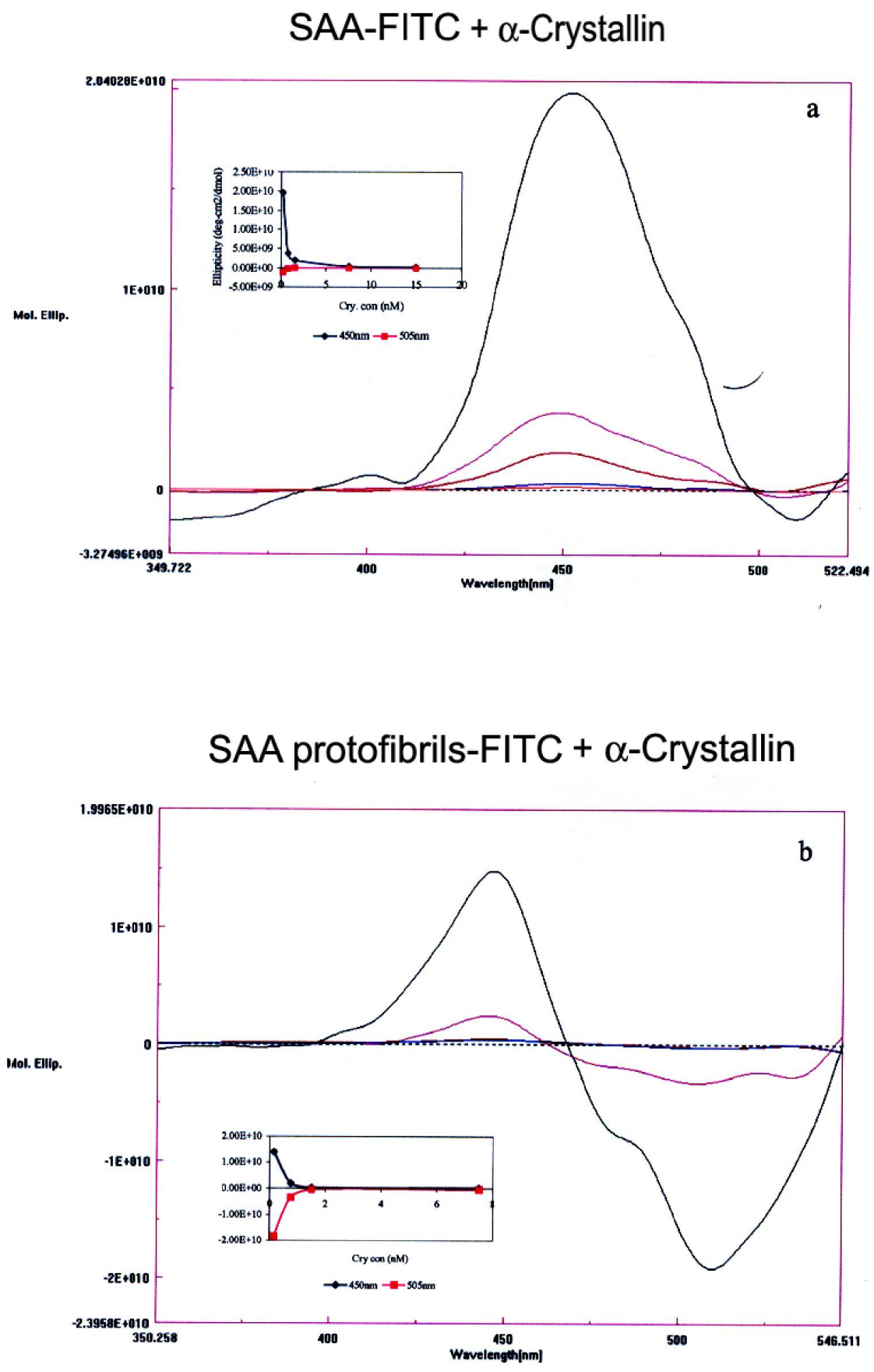


Fig. 3. CD spectra of SAA-FITC (a) SAA-FITC (2.5 mM) with increasing concentration of (0.15-15 nM) alphaB-Crystallin and insert plot shows concentration Vs ellipticity at 450 and 505 nm, (b) SAA-FITC protofibrils (2.5 mM) with increasing concentration (0.15-15 nM) alphaB-Crystallin and insert plot shows concentration Vs ellipticity at 450 and 505 nm

Further addition of αB-Crystallin did not change the ellipticity at 505 nm. FITC labeled SAA protofibril also showed similar effect on addition of αB-Crystallin. The CD band at 450nm gradually decreased as a function of αB-Crystallin (Fig. 3b). The negative band at 505 nm increased even at very low concentration of αB-Crystallin. Further addition (7.5 nM) did not change the ellipticity significantly. Flow cytometry revealed that the SAA-FITC positive binding readily occurred in the presence of αB-Crystallin in mouse BC3H1 brain cells and is shown in histograms. Both mean fluorescence intensity, as well as percentage of positive cell increased as a function of αB-Crystallin. However, the binding of SAA-FITC was almost completely prevented by simultaneous addition of αB-Crystallin. The histogram (Fig. 4a) showed the decrease in the percentage of positive binding than in the FITC-SAA control experiment during addition of αB-Crystallin. The mean fluorescence intensity was decreased to 45% of control values 55% in BC3H1 cells, respectively (Fig. 4a). The cell volume and granulating changes were also studied by using forward and side scatter plot. In all cells studied the forward scatter values were not varied significantly indicating that the volume of cells remain constant during αB-Crystallin addition. However, Side scatter values were increased significantly indicating that the αB-Crystallin treated cells were highly granular (Fig. 4b, c and d).

We next studied the cellular binding of SAA protofibril-FITC in the presence of various concentration of αB-Crystallin. The SAA protofibril-FITC binding to BC3H1 decreased markedly during the addition of αB-Crystallin. The mean fluorescence intensity decreased from 70% to 30% as shown in FL plot. From the histogram, the shifting of cell population from fluorescence positive region towards control cells (Fig. 5a) is seen.

DISCUSSION

The principal role of SAA during the acute-phase reaction appears to be the association with HDL-particles and subsequent changes of apolipoprotein composition and metabolic properties of its physiological carrier. Because SAA may displace apolipoprotein A-I, the major apolipoprotein of HDL, it is hypothesized that SAA could alter the protective function of HDL during 'reverse cholesterol transport (Artl 2000). SAA binding acute phase reaction was studied with macrophages (representative peripheral cells) revealed that the binding affinity for SAA is enhanced, Earlier findings suggest that extracellular matrix proteins appear to serve as a temporary anchorage sites for SAA and amyloid A (Preciado-Patt *et al.,* 1996).

The FITC fluorescence quenching ability of protein varies in proportion to disulfide groups. In monomeric SAA, addition of αB-Crystallin causes quenching of fluorescence. This clearly indicates that FITC environment in SAA is near to the disulfide groups of αB-Crystallin. However, the mechanism of binding of αB-Crystallin to SAA is still unknown. The SAA protofibril-FITC also interacts with αB-Crystallin resulting in enhanced fluorescence intensity. This is possibly due to exposure of FITC region of SAA to a more polar environment. In the case of the αB-Crystallin binding to protofibril, the FITC region is far away from the disulfide groups of αB-Crystallin. Decay of FITC labeled SAA showed multiple fluorescent lifetime species and addition of αB-Crystallin significantly decreased the average fluorescence lifetime of FITC. The decrease in lifetime of FITC is possibly due to the association of αB-Crystallin to the SAA. The interaction of disulfide groups in αB-Crystallin on FITC groups of SAA quenched the fluorescence of probe, which is accompanied by a decrease in fluorescence lifetime. From the induced CD studies, it is clear that the folding of SAA occurs up to 2 nM concentration of αB-Crystallin. Further addition of αB-Crystallin did not change the FITC region of SAA. However, in the protofibrils, FITC-environment changes were observed only upto 1 nM of αB-Crystallin. Futher addition of αB-Crystallin did not altered the conformation of FITC molecular environment indicating the completion of the folding process at low concentration of αB-Crystallin in protofibrillar than in native form of SAA.

Using flow cytometric studies, the competing nature of αB-Crystallin with monomeric and protofibrillar SAA binding was studied in BC3H1 cells. αB-Crystallin did not compete with monomeric SAA binding to BC3H1 cells. This is possibly due to the occurrence of a large number of high affinity SAA binding sites on BC3H1 cells. However, BC3H1 cells the SAA binding decreased in the presence of αB-Crystallin. This effect is either due to the presence of low affinity SAA binding site or competing effect of αB-Crystallin with SAA in the binding process. αB-Crystallin may prevent the SAA mediated toxic mechanism in BC3H1. Studies shown in BC3H1 CELLS increases in both muscle protein degradation and levels of mRNAs encoding components of the ubiquitin–proteasome pathway have been found in association with other catabolic conditions, including sepsis, cancer, burns, starvation, and denervation. onsequently, insights into the mechanisms leading to increased mRNAs for components of this pathway could have widespread interest (Bailey *et al.,* 1996). The metabolic acidosis causes infants and children to grow poorly, and showed that induction of metabolic acidosis in normal adults by administration of ammonium chloride accelerated catabolism of both protein and branched-chain amino acids (BCAAs). Experimental metabolic acidosis in rats reduces growth and stimulates whole body proteolysis and BCAA catabolism (Isozaki *et al.,* 1971).

Protofibril binding to BC3H1 cells significantly decreased in the presence of αB-Crystallin. This effect may be either due to αB-Crystallin complexation of SAA protofibrils. The αB-Crystallin incubation with SAA significantly increased the granularity in cell lines. Endocytosed proteins and most membrane components are degraded by another proteolytic system, acidic proteases in lysosomes, but this organelle also appears to be important in degrading certain cytosolic proteins, especially in catabolic states (Mitch *et al.,* 1994). The earlier study showed the morphological, physiological, and biochemical analyses raised the possibility that BC3H1 cells were of smooth muscle origin. The observation of vascular smooth muscle a-actin in BC3H1 myocytes supports the smooth muscle classification. since previous reports have shown that the expression of this actin isoform can be used diagnostically as a specific biochemical marker for vascular smooth muscle cells (Strauch *et al.,* 1986).

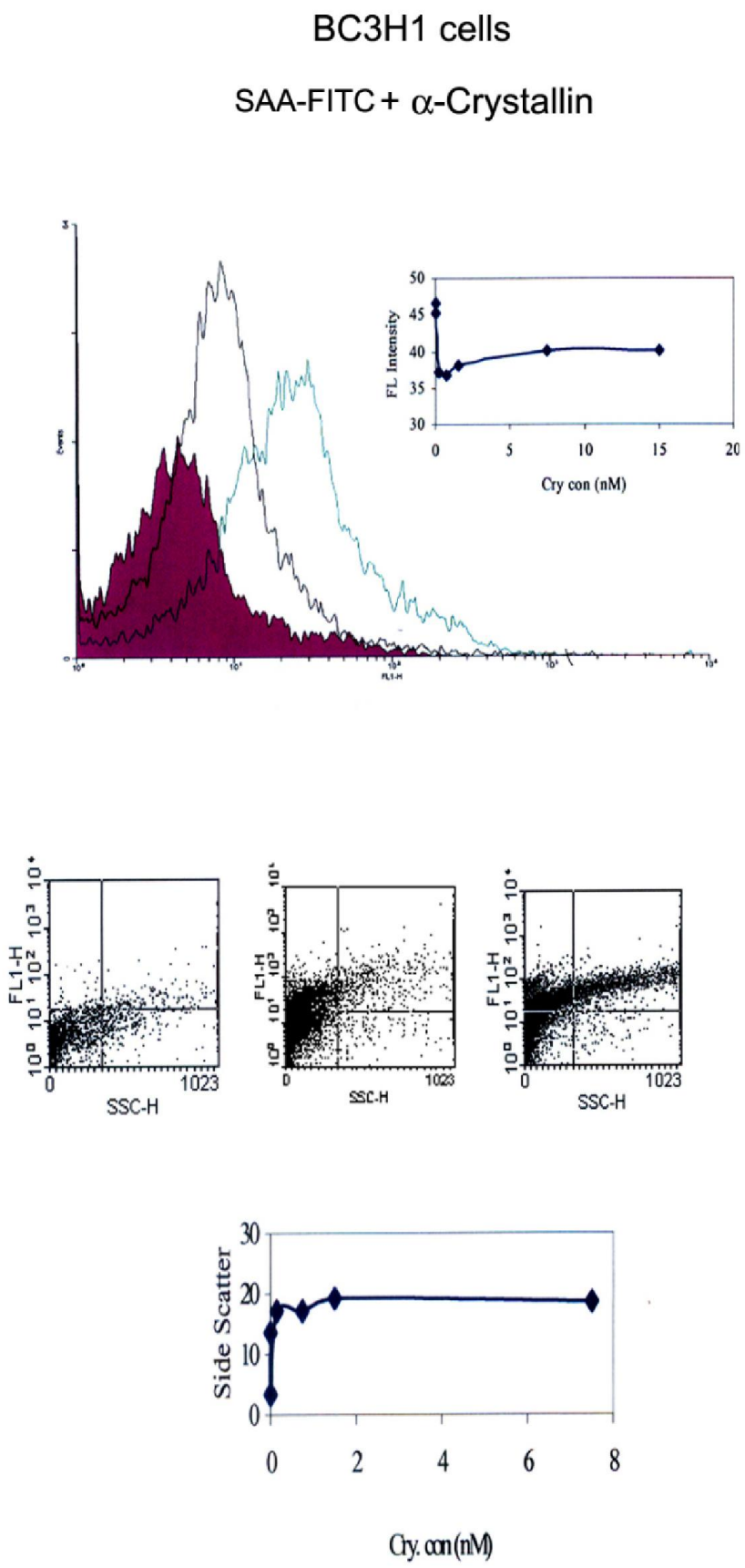


Fig. 4. Effect of alphaB-Crystallin on SAA-FITC binding to mouse BC3H1 cells (a) histogram shows filled curve control cells, black curve (6.25 mM) SAA-FITC, green curve shows addition of (0.75 nM) alphaB-Crystallin and insert plot shows increasing concentration of alphaB-Crystallin (0.15-15 nM) Vs mean fluorescence (b) side scatter dot plot of BC3H1 cells and incubated with SAA-FITC (2.5 mM) (c) SAA-FITC with (0.15-15 nM) alphaB-Crystallin, (d) plot shows alphaB-Crystallin concentration Vs side scatter (upper right)

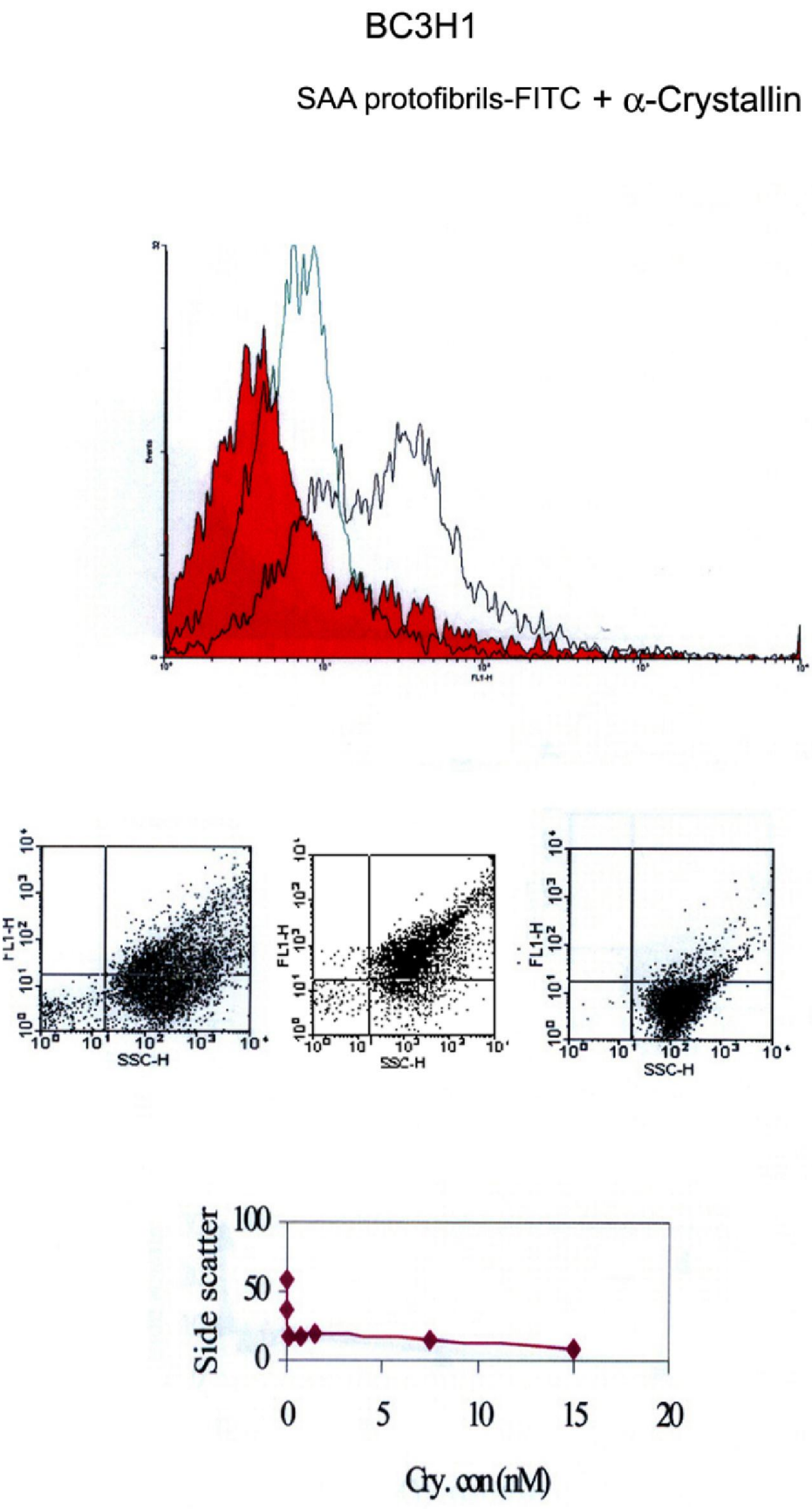


Fig. 5. Effect of alphaB-Crystallin on SAA protofibrils-FITC binding to mouse BC3H1 cells (a) histogram shows filled curve control cells, black curve (6.25 mM) SAA protofibrils-FITC, green curve shows addition of (0.75 nM) alphaB-Crystallin and insert plot shows increasing concentration of alphaB-Crystallin (0.15-15 nM) Vs mean fluorescence (b) side scatter dot plot of BC3H1 cells and incubated with SAA protofibrils-FITC (2.5 mM) (c) SAA protofibrils-FITC with (0.15-15 nM) alphaB-Crystallin, (d) plot shows alphaB-Crystallin concentration Vs side scatter (upper right)

Conclusion

In conclusion, the interaction and binding of SAA protein and porotofibrils with BC3H1 cells decreases in the presence of αB-Crystallin and the cytotoxicity of the SAA and SAA protofibrils reduced.

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REFERENCES

Arrigo, A. P. and Landry, J. The biology of heat-shock proteins and molecular chaperones (Morimoto, R., Tissieres, A., and Georgopoulus, C., Eds.) (1994) pp 335-373, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Artl, A., Marsche, G., Lestavel, S., Sattler, W. and Malle, E. Role of serum amyloid A during metabolism of acute-phase HDL by macrophages. Arterioscler Thromb Vasc Biol. 20 (2000) 763-72.

Behlke, J., Lutsch, G., Gaestel, M., and Bielka., H. Supramolecular structure of the recombinant murine small heat shock protein hsp25. FEBS. Lett. 288 (1991) 119-122.

Burton Wice, Jeffrey Milbrandt, and Luis Glaser. 1987. Control of Muscle Differentiation in BC3H1 Cells by Fibroblast Growth Factor and Vanadate. THE J. Biol. Chem. 262, 1810-1817.

Caspers, G. J., Leunissen, J. A. and de Jong, W. W. J. 1995. The expanding small heat-shock protein family, and structure predictions of the conserved "alpha-crystallin domain". Mol. Evol. 40, 238-248.

De Jong, W. W., Leunissen, J. A. M., and Voorter, C. E. M. 1993. Evolution of the alpha-crystallin/small heat-shock protein family. Mol. Biol. Evol. 10, 103-126.

Groenen, P. J. A., Merck, K., de Jong, W. W., and Bloemendal, H. 1994. Structure and modifications of the junior chaperone alpha-crystallin. From lens transparency to molecular pathology. Eur. J. Biochem. 225, 1-19.

Horwitz, J. 1993. A small heat shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state. *Invest. Ophthalmol. Vis. Sci.,* 34, 10-22.

Horwitz, J. 1992. Alpha-crystallin can function as a molecular chaperone. Proc. Natl. Acad. Sci. U.S.A. 89, 10449-10453.

Isozaki U, Mitch WE, England BK, Price SR. Protein degradation and increased mRNAs encoding proteins of the ubiquitin-proteasome proteolytic pathway in BC3H1 myocytes require an interaction between glucocorticoids and acidification. Proc Natl Acad Sci U S A. 93 (1996) 1967-71.

Kaplan, B., Hrncic, R., Murphy, C. L., Gallo, G., Weiss, D. T., and Solmon, A. 1999. Micro extraction and purification techniques applicable to chemical characterization of amyloid proteins in minute amounts of tissue. Methods. Enzymol. 309, 67-81.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227 680-685.

Lathrop B, Olson E, Glaser L. Control by fibroblast growth factor of differentiation in the BC3H1 muscle cell line. J Cell Biol. 100 (1985) 1540-7.

Lee, G. J., Pokala, N. and Vierling, E. 1995. Structure and in vitro molecular chaperone activity of cytosolic small heat shock proteins from pea. J. Biol. Chem. 270, 10432-10438.

Lee, G. J., Roseman, A. M., Saibil, H. R., and Vierling E. A. 1997. 1997. Small heat shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state. EMBO J. 16, 659-671.

Lindhorst, E., Young, D., Bagshaw, W., Hyland, M., Kisilevsky, R. Biochim. Biophy. Acta. 1339 (1997) 143-154.

Linke, R. P., Bock, V., Valet, G. and Rothe, G. 1991. Inhibition of the oxidative burst response of N-formyl peptide-stimulated neutrophils by serum amyloid-A protein. *Biochem. Biophys. Res. Commun.,* 176, 1100-1105.

Lowry Oh, Rosebrough Nj, Farr Al and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* 193, 665-675.

Luthra, M. and Balasubramanian, D. 1993. Nonenzymatic glycation alters protein structure and stability. A study of two eye lens Crystallin. J. Biol. Chem. 268, 18119-18127.

Merck, K. B., Groenen, P. J. T. A., Voorter, C. E. M., de Haard-Hoekman, W. A., Horwitz, J., Bloemendal, H., and de Jong W. W. J. Biol. Chem. 268 (1993) 1046-1052.

Parsell, D. A. and Lindquist, S. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu. Rev. Genet. 27 (1993) 437-496.

Preciado-Patt, L., Levartowsky, D., Pras, M., Hershkoviz, R., Lider, O., and Fridkin, M. Inhibition of cell adhesion to glycoproteins of the extracellular matrix by peptides corresponding to serum amyloid A. Toward understanding the physiological role of an enigmatic protein. Eur. J. Biochem. 223 (1994) 35-42.

Rousset Vincent Kindler, Conrad Hauser Jean-François Arrighi, and Michela Rebsamen Françoise. 2001. Lipopolysaccharide, TNF- a, and Contact Blood-Derived Dendritic Cells Induced by Protein Kinase in the Maturation of Human A Critical Role for p38 Mitogen-Activated. *J. Immunol.,* 166, 3837-3845.

Strauch AR, Offord JD, Chalkley R, Rubenstein PA. Characterization of actin mRNA levels during BC3H1 cell differentiation. J Biol Chem. 261 (1986) 849-55.

Wistow, G. 1993. Possible tetramer-based quaternary structure for alpha-crystallins and small heat shock proteins. Exp. Eye Res. 56, 729-732.

Xu, L., Badolato, R., Murphy, W. J., Longo, D. L., Anver, M., Hale, S., Oppenheim, J. J., and Wang, J. M. 1995. A novel biologic function of serum amyloid A. Induction of T lymphocyte migration and adhesion. *J. Immunol.,* 155, 1184-1190.

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