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Heat Shock Proteins of Chicken Lens

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Abstract The presence of heat shock proteins HSP-40, HSP-70, and HSc-70 in adult and embryonic chicken lenses were determined. The epithelium, cortex, and nucleus of adult chicken lens were separated and tested for the presence of heat shock proteins (hsps) by western blot, using specific antibodies for HSP-40, HSP-70, and HSc-70. Water soluble (WSF) and water insoluble fractions (WIF) of embryonic chicken lenses were isolated and tested for the presence of HSP-40, HSP-70, and HSc-70 by immunoblot. Embryonic chicken lens sections were also analyzed for the presence of heat shock proteins by immunoflorescence technique. Data obtained from these experiments revealed that HSP-40, HSP-70, and HSc-70 are present in all areas of both adult and embryonic chicken lens. Presence of hsps protein in the deep cortex and nucleus is intriguing as no detectable metabolic activities are reported in this area. However it can be proposed that hsps HSP-40, HSP-70, and HSc-70 can interact with protein of these areas and protect them from stress induced denaturation. J. Cell. Biochem. 82: 409–414, 2001.

Key words: chicken adult and embryonic lens; HSP-40; HSP-70; HSc-70; immunoblot; heat shock proteins

Maintenance of the native tertiary organization of proteins is of paramount importance for transparency of the ocular lens. Interference with the integrity of its constituent protein structure can induce cataract. The native three dimensional organization of proteins in both prokaryotes and eukaryotes is preserved by a special group of molecules named chaperones or heat shock proteins [Becker and Craig, 1994; Morimoto et al., 1994]. The chaperones maintain the native structure of all proteins and also participate in (a) the proper folding of newly synthesized polypeptides, (b) the movement of secretory proteins through the endoplasmic reticulum, and (c) in the degradation and digestion of unfolded or partially degraded proteins [Eisenberg, 1999]. In the lens, α_A or α_B, crystalines behave like chaperones and have been shown to prevent aggregation of lens proteins [Klemenz et al., 1991; Horwitz, 1992; Lee et al., 1993]. However, these small heat shock proteins cannot refold denatured proteins to their native states or protect newly synthesized proteins from misfolding. These chaperone activities are performed by ATP-assisted higher molecular weight heat shock proteins [Hendrick and Ulrich Hartl, 1995].

Even without stress newly synthesized polypeptides can aggregate nonspecifically with other polypeptides, in the absence of specific molecular chaperones such as HSP-70 and HSP-40 [Becker and Craig, 1994]. Thus, molecular chaperones are essential for the maintenance of native protein structure during its synthesis, and for protecting it from stress-induced destablization.

The lens provides a unique model system to examine the role of different hsps in the maintenance of native protein organization. The anterior epithelium has a relatively high rate of protein synthesis, whereas differentiating equatorial fiber cells have a lower rate of protein synthesis and no detectable synthesis has been reported in the nuclear region of the lens. Thus the role of hsps in the maintenance of newly synthesized and long lived proteins can be analyzed in one tissue.

In this study, we determined the distribution of hsps HSP-70, HSc-70, and HSP-40 in discrete regions of both adult and embryonic chicken lenses. These experiments revealed that hsps are present in both adult and embryonic lenses and that they are distributed in all regions of the lens.

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MATERIALS AND METHODS

Adult White Leghorn chicken eyes were obtained from a local poultry immediately after sacrifice and the lenses removed. The lenses were dissected to separate annular pad cell, cortical fibers, and nuclear fibers. Water soluble (WSF) and water insoluble fraction (WIF) were isolated from each lens region as reported earlier [Katar et al., 1993] and dissolved in 2% SDS containing buffer. Fertilized eggs of White Leghorn chickens were incubated at 37.5°C and removed at specific times for the isolation of lenses. All antibodies were obtained from Stressgen (Victoria, B.C. Canada) and immunoblot assays in our laboratory confirmed that HSP-70 (1), HSc-70 antibodies only recognize their specific proteins as claimed by the vendor.

Lens tissues were fractionated by 10% SDS–PAGE technique, and electroblotted to nitrocellulose paper [Atreya et al., 1989]. The nitrocellulose strips were blocked with 1% albumin solution and exposed to primary antibody. Alkaline phosphatase labelled IgG (BioRad) were used as secondary antibody and color was developed using Bio-rad reagents.

Immunoflorescence

Eleven day old chick embryo eyes or lenses were fixed in formaldehyde, embedded in paraffin, and sectioned. The sections were exposed to specific polyclonal or monoclonal antibodies and after washes reacted with FITC conjugated secondary antibody [Waggoner and Maisel, 1978]. Sections were examined with a Zeiss axiomat epifloresence microscope and digitized images captured with metamorph software.

RESULTS

Experiments were performed to look for the presence of HSP-70 (inducible), heat shock cognate HSc-70 and HSP-40 in adult lens epithelium, cortex, and nucleus. Figure 1A shows the SDS–PAGE pattern of the water-insoluble fractions (WIF) of the lens, and purified proteins HSP-70, HSP-40, and HSc-70. Each region of the lens contained a single protein which cross-reacted with the polyclonal antibody to HSP-70 (Fig. 1B). The same result was obtained with the monoclonal antibody to Hsc-70 (Fig. 1C), and with the polyclonal antibody

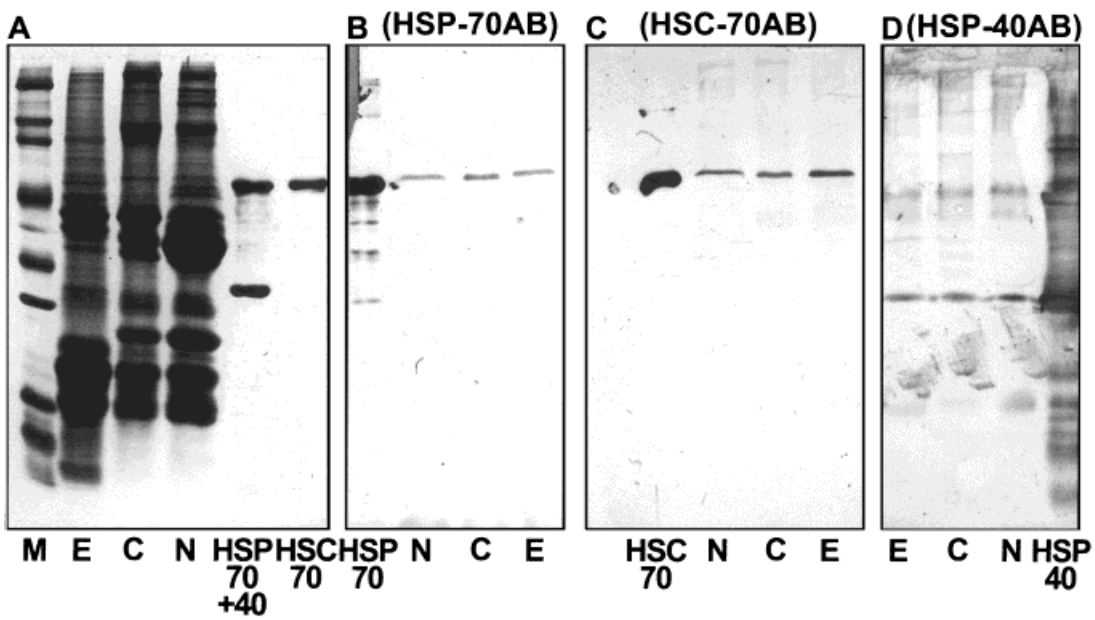

Fig. 1. Freshly isolated adult chicken lenses were dissected to separate epithelium, cortex, and nucleus. All tissues were dissolved in SDS containing buffer and fractionated by SDS–PAGE. Four sets of gels were run. One set was stained with Coomassie blue (**A**) and the other three were used for immunblot (**B, C** and **D**). M = Molecular weight markers, E = Epithelium, C = Cortex and N = Nucleus. 70 (1) = HSP-70, 70 = Hsc-70, and 40 = HSP-40.

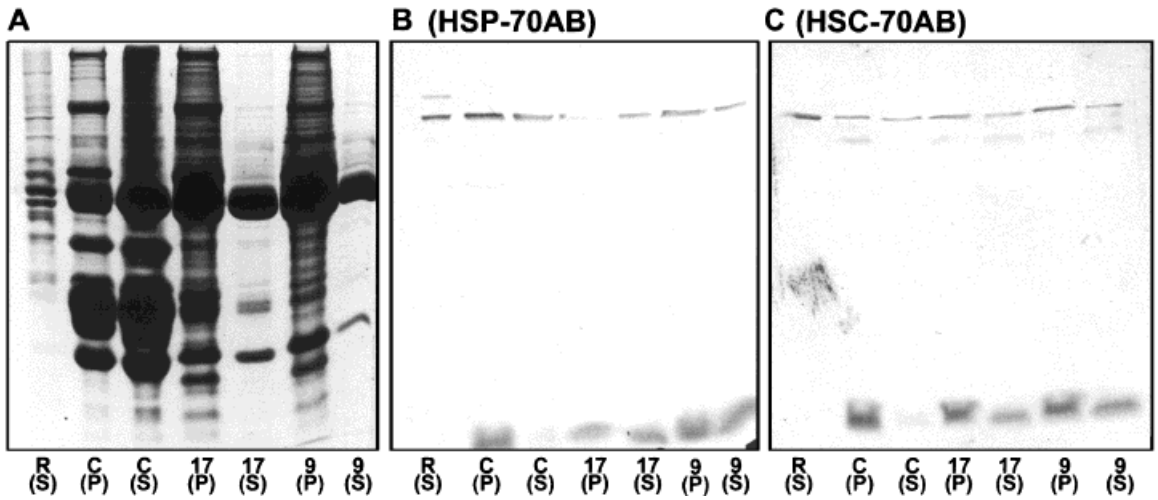

Fig. 2. SDS–PAGE of embryonic and adult chicken lens soluble and insoluble proteins. 9 = 9 day old embryonic (E) chicken lens. 17 = E-17 lens, S = soluble protein, P = water insoluble protein, C = adult chicken, R = adult chicken retina, 2A = Coomassie blue stained protein profile of adult and embryonic chicken lens, 2B = Immunoblot of 2A with HSP-70 (1) antibody, and 2C = Immunoblot of 2A with Hsc-70 antibody.

HSP-40 (Fig. 1D). Thus HSP-70, HSc-70, and HSP-40 were present in all regions of the lens.

The distribution of heat shock proteins in the water-soluble fraction (WSF) and water-insoluble fraction (WIF) of the embryonic chicken lens was also determined. Figure 2 shows that HSP-70 and HSc-70 were present in both soluble and insoluble fractions of E-9 and E-17 lenses, and adult cortex. Adult chicken retina also contains HSP-70 and HSc-70 as previously reported [Morales et al., 1998]. Figure 3 shows the presence of HSP-40 in embryonic lens in both soluble and insoluble fractions. In the neural retina HSP-40 appeared as a doublet. Immunoflorescence showed a distribution of HSP-70 in all regions of the 11 day embryonic lens, as well as in the iris–ciliary complex and endothelial layer of the cornea. (Fig. 4A) HSc-70 and HSP-40 showed similar distribution (Fig. 4B and C). Negative controls were incubated with rat IgG (Sigma) or mouse ascites

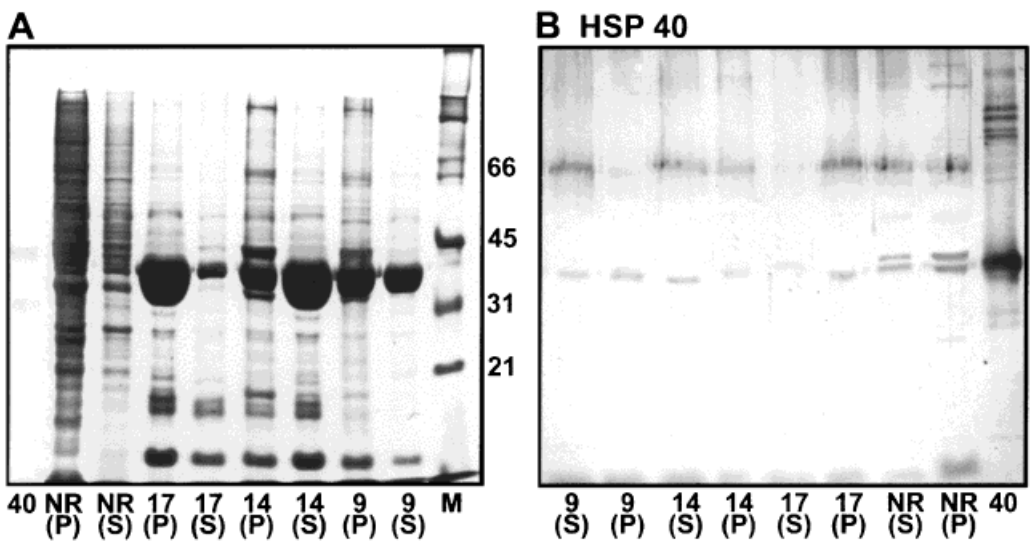

Fig. 3. SDS–PAGE profile of embryonic chicken lens and neural retina proteins. 9 = E-9, 14 = E-14, 17 = E-17, and NR = E-17 neural retina, 40 = HSP-40, 3A = Coomassie blue stained protein profile of embryonic chicken lens and neural retina, and 3B = Immunoblot of 3A with HSP-40 antibody.

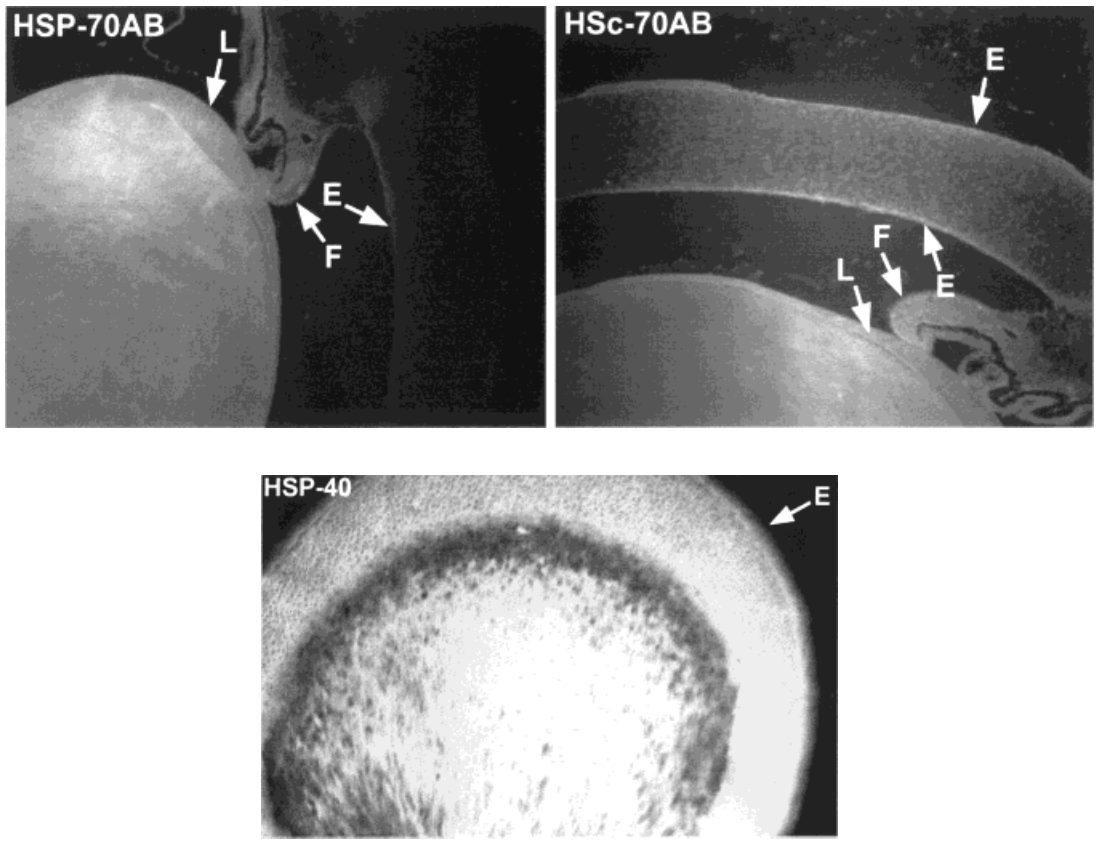

Fig. 4. Distribution of HSP-70 (4A), Hsc-70 (4B), and HSP-40 (4C) immunoreactivity in E-12 lens, cornea, and ciliary body. L = Lens epithelium, F = Zonule fiber, and E = Cornea and ciliary body.

fluid (Sigma), and no immunostaining were detected.

DISCUSSIONS

Horwitz [1992] demonstrated that lens α_B and α_A crystallins act as small heat shock proteins (shsps) to prevent stress induced denaturation of lens proteins. It has also been reported that in response to stress, shsps are the most strongly induced heat shock proteins [Inaguma et al., 1992]. However, shsps by themselves cannot refold partially denatured polypeptides, or prevent newly synthesized polypeptides from non-specific aggregation. Thus the major role of α-crystallins may be to sequester denatured lens proteins and prevent their aggregation [Merck et al., 1993]. It has also been reported that chaperones of the HSP-70 family (Dnak) in collaboration with proteins of HSP-40 family (DnaJ) refold partially denatured proteins or prevent the non-specific aggregation of newly synthesized polypeptides [Hendrick and Ulrich Hartl, 1995; Pierpaoli et al., 1997].

Constitutively expressed HSc-70 and stress inducible HSP-70 are the predominant hsps of the HSP-70 family [Fourie et al., 1994; Freeman et al., 1995]. Both proteins can bind unfolded proteins and require ATP for their biological functions. HSP-70 is stress inducible, whereas HSc-70 is present in physiologically stable cells and participates in many normal biological activities such as protein translocation and protein synthesis, HSP-70 also requires other chaperones such as HSP-40 and HSP-90 for its activity. Results of our experiments showed that HSc-70, HSP-70, and HSP-40 are present in all regions of the adult and embryonic chicken lens.

It is evident from Figure 1 that HSP-70 and HSP-40 are present in nearly equal amount (per mg lens protein) in the epithelium, cortex, and nuclear areas of the lens, whereas HSc-70 in comparatively higher in the epithelium of the

adult chicken lens. These results suggest that all of the above mentioned hsps are involved in the maintenance of protein integrity in unstressed cells. It is puzzling to find HSP-70, HSc-70, and HSP-40 in the nucleus of the lens, as these proteins require ATP for their activities and the lens nucleus is not known to contain abundant ATP. Moreover HSP-70 and HSP-40 collaborate in the maintenance of proper protein folding, and the attachment of HSP-40 to HSP-70 is dependent on ATP hydrolysis. Thus in the chicken lens cortex and nucleus, where ATP is sparse, these molecular chaperones may protect proteins from degradation in an ATP independent fashion. In human, bovine, and calf lenses, HSc-70, HSP-70, and HSP-40 are only present in the epithelium and superficial cortical fiber cells (personal observation). This difference could be due to specificity of avain lens hsps, as their proteins are stable and not prone to age related cataract or hsps of chicken lenses act both as refolder and stabilizer of the denatured proteins. Thus the presence of molecular chaperones in the chicken lens nucleus is intriguing. The presence of higher amounts of HSc-70 in the chicken lens epithelium suggests a role in protein folding and translocation, as these cells have active protein synthetic activities.

HSP-70, HSc-70, and HSP-40 are present in both soluble and insoluble fractions of the embryonic chicken lens. The amounts of heat shock proteins in both soluble and insoluble fractions are similar so it can be assumed that soluble hsps that were free, unbound proteins, whereas the insoluble fraction is part of a protein complex. It can be proposed that in chicken lenses above mentioned hsps participate in both ATP mediated refolding and also stabilizing denatured proteins. In refolding, after establishment of native state of denatured proteins, hsps are released, whereas stabilizing hsps remain bound with the partially denatured proteins, thus presence of free and bound hsps in the lens cell cytosol signify two specific roles of the hsps. As protein synthesis and cell division is higher in embryonic lenses compared to the adult lens, the presence of significantly higher concentration of molecular chaperones was expected [Collier and Schlesinger, 1986; Dash et al., 1994; Tanaka et al., 1995; Nakamura et al., 2000]. However, the data presented here do not support that. It is interesting to note that both adult chicken cortical soluble and insoluble fractions contain similar amounts of heat shock proteins as that of embryonic lens soluble and insoluble proteins.

Immunoflorescence studies of the embryonic chicken lens confirmed that all hsps are present in all areas of the embryonic chicken lens.

Molecular chaperones are ubiquitous and their presence in adult and embryonic chicken lenses suggest that lens proteins like all other cellular proteins, require both regular molecular chaperones, namely HSP-70, HSc-70, HSP-40, and the shps like α-crystallins to maintain its integrity. It has been reported that with aging or chronic stress, molecular chaperone activity decreases, and this decrease could cause disorganization of protein structure and eventual lens opacities [deJong et al., 1986; Inaguma et al., 1992; Morales et al., 1998; Dean et al., 1999; Singh et al., 1999]. However hsps of adult chicken lenses did not show any appreciable decrease in comparison to embryonic tissues, it is possible that hsp levels in the chicken lens does not decrease significantly until they are very old. Presently, we are examining lenses of aged chickens.

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