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Research Notes

A Novel 29-kDa Chicken Heat Shock Protein

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ABSTRACT The family of small heat shock proteins is the more variable among the highly conserved superfamily of heat shock proteins (HSP). Using a metabolic labeling procedure with tissue explants, we have detected in chickens a new member of the small HSP family with an apparent molecular weight of 29-kDa. This protein was induced in broiler chickens’ heart muscle and lungs following an in vivo heat stress. The 29-kDa band appears after 3 h of heat stress, much later than the induction of HSP 90, HSP 70, and HSP 27. The late onset of induction suggests that HSP 29 plays a more specific role of a “second stage defense protein.”

(Key words: chicken, heat shock proteins, heat stress, metabolic labeling)

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INTRODUCTION

Heat shock proteins (HSP), also known as stress proteins, are among the most conserved proteins known in phylogeny with respect to both function and structure (reviewed in Lindquist, 1986; Gething and Sambrook, 1992; Jaattela and Wissing, 1992; Caspers et al., 1995). They act as molecular chaperones by binding to other cellular proteins and assisting their folding into the proper secondary structures, thus preventing misfolding and aggregation during stress.

The superfamily of HSP includes a number of different molecular weight class families: HSP 110, HSP 90, HSP 70, HSP 60, HSP 47, and a group of small HSP ranging from 16 to 40 kDa in various species (Lindquist and Craig, 1988; White et al., 1994). This evolutionary variable subfamily of proteins is characterized, however, by the presence of a conserved homologous “α-crystalline domain” (Ingolia and Craig, 1982) that is present sometimes in duplicate in the protein metabolite. Predictions of that secondary structure and solvent accessibility of this domain, together with hydropathy profiles and intron positions, support the presence of two similar hydrophobic β-sheet-rich motifs connected by a hydrophilic α-helical region.

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FIGURE 1. Autoradiogram showing induction of heat shock proteins (HSP). Broiler chickens were subjected to high ambient temperature at the age of 42 d. Samples of lung (A), and heart muscle (B), homogenates containing equal amounts of trichloroacetic-acid-precipitable radioactivity were applied to a 10% SDS-PAGE gel. Lanes 1 to 5 represent 0, 30, 60, 180, and 240 min exposure to 37.2 C. Lane 6 represents 48 h recovery from 240 min stress. M = molecular size markers.

Lyophilized buffer (Laemmli, 1970), heat-denatured at 95 C for 5 min and centrifuged at 10,000 x g and 4 C for 10 min. The supernatants were collected and aliquots were stored at –20 C.

The radioactivity of the radiolabeled cellular proteins was measured by spotting 5 µL from each sample onto Nitrocellulose filters6 in duplicate or triplicate. The cellular proteins were precipitated on the filters by 15 min incubation at 4 C in 10% trichloroacetic acid (TCA)5 followed by boiling in 5% TCA for 10 min and washed with ethanol before counting in a beta counter. Differences between duplicates were up to 5%.

The cellular proteins were separated by one-dimension SDS-PAGE (Laemmli, 1970). The amount of proteins loaded on the gels was based on the radioactivity. An equal amount of radioactivity (3 x 10^4 cpm) was loaded in each lane. Gels containing radiolabeled proteins were processed for fluorography, dried in a SE1160 gel dryer7 and exposed for 4 d to Kodak X-Omat AR film8 for autoradiography. Molecular weight markers were [14C]Rainbow markers.9

RESULTS AND DISCUSSION

As shown by the electrophoresis pattern (Figure 1), an acute 4-h exposure of broiler chickens to 37.2 C (with 20 to 30% relative humidity) resulted in enhanced synthesis of three major HSP in heart muscle and lungs. The synthesis rate of HSP 90, HSP 70, and HSP 27 accelerated gradually. Maximal rates of synthesis were detected after 4 h, which was the longest duration of heat exposure tested. At this time point most chickens were dying and body temperatures were 47 C ± 0.5. The synthesis of all HSP was hardly detectable in chickens not exposed to the temperature stress. Among the HSP whose rate of synthesis increased, a previously unknown HSP of about 29 kDa (HSP 29) was detected. Synthesis of the newly identified HSP 29 became apparent only after 3 h of exposure to heat stress. It could be detected in lungs (Figure 1A), in heart muscles (Figure 1B) and in peripheral blood leukocytes (not shown).

The novel HSP 29 had not been detected previously in peripheral leukocytes of turkeys (Wang and Edens, 1993) and broiler chickens (Edens et al., 1992) in which high ambient temperature had been applied both in vitro and in vivo for up to 120 min. This protein was also not detected in cultured skin fibroblasts of broiler chicken (Einat and Yahav, unpublished data) and in heat-stressed cultured chick pineal cells (Wolf and Zats,
In these cases, the failure to detect HSP 29 could be explained by genetic variation, tissue specificity, or the relatively longer time period required for its induction. For initial clarification of this question, peripheral blood lymphocytes were prepared from broiler chickens subjected to high ambient temperature for 6 h using the same protocol as Edens et al. (1992) and HSP 29 was detected (not shown). Edens et al. (1992) did not detect HSP 29 in peripheral lymphocytes of heat-stressed broiler chickens using a heat-stress challenge at a much higher temperature (43 °C) but for a shorter duration (60 min). Therefore, lacking information on cloacal temperature, we cannot determine whether their failure to detect HSP 29 is due to a milder hyperthermia of the broiler chickens or to the small difference in genetic background (Arbor Acres vs Cobb).

The difference in induction time of HSP 29 suggests that this protein is regulated by a mechanism different than that involved in the induction of other HSP such as HSP 90 and HSP 70. A relatively late onset of induction was observed previously in chickens also for HSP 23 (Lindquist and Craig, 1988; Edens et al., 1992) and in mammals for other small HSP (reviewed by Arrigo and Landry, 1994). These low molecular weight HSP, therefore, may represent an advanced stage defense mechanism activated only when stress becomes severe. Small HSP of around 30 kDa were reported in some cases such as chicken mononuclear cells (HSP 32, Miller and Qureshi, 1992) and chicken chondrocytes (HSP 31, Neri et al., 1992). It is possible that these proteins and HSP 29 are related and that variability in structure is due to differential posttranslational modifications. Post-translational modifications of HSP isoforms were shown previously for the human HSP 30 family (Cretien and Landry, 1988). Another possibility is that these proteins differ in their primary structure as was shown previously for the family of the fish HSP 30 family (White et al., 1994).

The procedure used in the present study has been found effective in detecting heat stress response at the level of protein synthesis. HSP 29 and other proteins that could be found using our protocol may provide markers for genetic breeding towards a more heat-stable strain of chicken.

REFERENCES


