Volume 289, number 1, 110–112 FEBS 10114 September 1991

ADONIS 0014579391008305

Different expression time of the 105-kDa protein and 90-kDa heat-shock protein in rat testis

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Received 27 June 1991

To understand the physiological functions of the 105-kDa protein which is testis-specific and HSP90 (90-kDa heat-shock protein) related protein, the appearance of it in the testis has been followed during the development of rat. On immunoblotting analysis, the 105-kDa protein did not appear until after the age of five weeks, while HSP90 could be detected at three weeks. In the spermatozoa, the 105-kDa protein was much abundant but not in the LC-540 cells (a cell line from Leydig cell tumor in rat testis) cytosol. This finding has attracted much attention to the relationship between this protein and sperm functions.

Heat-shock protein; Stress protein; HSP90; 105-kDa Protein

1. INTRODUCTION

A wide variety of environmental stresses induces cells to rapidly synthesize a group of polypeptides known as heat-shock proteins (stress proteins) [1]. Among the heat-shock proteins, HSP90 is a cytoplasmic protein in unstressed cells. It has been shown that HSP90 is associated with steroid hormone receptors and regulates their activation mechanisms [2]. However, the physiological roles of heat shock proteins including HSP90 still remain to be elucidated.

Recently, we have reported that HSP90 was abundant in the brain and testis, and the antibody against HSP90 was cross-reacted with the newly observed 105-kDa protein as well as HSP90 [3]. The 105-kDa protein was detected only in the testis and the protein was not cross-reacted with anti-HSP100 antibody. The physicochemical properties, except the molecular mass, of the 105-kDa protein were similar to those of HSP90 and the protein seems to be a cognate protein of HSP90.

In order to understand the biological functions of the 105-kDa protein, we have investigated when the 105-kDa protein and HSP90 appear in developing rat testes.

Abbreviations: HSP90 and HSP100, 90-kDa and 100-kDa heat-shock protein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride; MEM, Eagle's minimum essential medium

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2. MATERIALS AND METHODS

2.1. *Antibody*

In this study, we used an antibody against bovine brain HSP90 as previously reported [3].

2.2. *Sample preparation*

Wister rat tested (3-, 4-, 5-, 6-, and 10-week-old) were homogenized with buffer A (10 mM Tris-HCl, 0.125 M NaCl, pH 7.4) and centrifuged at 18 000 × *g* for 20 min. The supernatants (crude extract) were used for SDS-PAGE and immunoblotting. The crude extract of Wister rat uterus and ovarium (10-week-old) were obtained by the same method. Rat epididyms (10-week-old) were cut with a scalpel. A lump of spermatozoa was collected into a Petri dish, washed twice with buffer A, and was collected by centrifugation at 2000 × *g* for 10 min. The pellet was checked for spermatozoa by microscope. The purified spermatozoa were sonicated and centrifuged at 18 000 × *g* for 10 min. The supernatant was used as spermatozoa cytosol.

2.3. *Cell culture*

LC-540 cells were purchased from American Type Culture Collection. The cells were grown in MEM supplemented with 10% fetal calf serum in a culture flask (Corning) at 37°C in a humidified atmosphere containing 5% CO_2. Upon reaching confluence, the cells were harvested and washed twice with buffer A. After centrifugation at 2000 × *g* for 10 min, the cells were collected. The pellet was then sonicated and centrifuged at 18 000 × *g* for 10 min. The supernatant was used for SDS-PAGE and immunoblotting.

2.4. *Gel electrophoresis*

SDS-PAGE was carried out by the procedure of Laemmli [4]. Gels were stained with Coomassie brilliant blue (R250) in a mixture of 25% isopropyl alcohol–10% acetic acid and destained with 10% isopropyl alcohol–10% acetic acid.

2.5. *Immunoblotting*

Samples were electrophoresed on SDS-polyacrylamide gels, transferred to PVDF membrane (Nihon Millipore Kogyo, Yonezawa, Japan) electrophoretically, and processed as described by Towbin et al. [5]. The membrane was incubated with anti-bovine brain HSP90 antibody (1:1000 dilution) and treated with horseradish peroxidase conjugate anti-rabbit IgG (1:1500 dilution) (Bio-Rad, Richmond,

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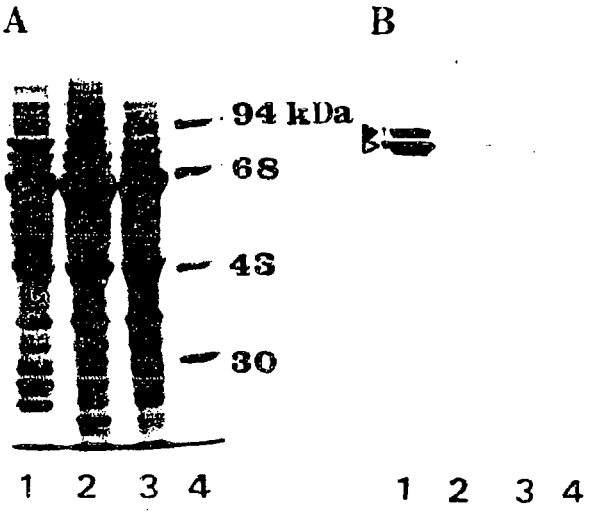

Fig. 1. Immunological detection of rat tissues HSP90. Wister male and female rat tissues (10-week-old) were homogenized with 10 mM Tris-HCl, 0.125 M NaCl (pH 7.4). The cytosols were electrophoresed (100 μg protein per lane) on SDS-polyacrylamide gel (9%). The gels were stained with Coomassie brilliant blue (panel A) or electrophoretically transferred to PVDF membrane and stained with anti-bovine brain HSP90 antibody (panel B). In both panels: lane 1, testis; 2, uterus; 3, ovarium; and 4, standard marker proteins (molecular mass given in kDa at side). The closed and open triangles indicate the 105-kDa protein and HSP-90, respectively.

CA). The peroxidase substrate was 3,3-diaminobenzidine tetrahydrochloride. The densities of the immunochemically stained bands were measured using a Hoffer scanning densitometer as previously described [6].

3. RESULTS

The immunoblotting method using anti-HSP90 antibody showed two bands in rat testis (Fig. 1). The slower and faster moving bands corresponded to 105 kDa and 90 kDa, respectively. The 105-kDa protein could not be detected in the other tissues when tested even at three times higher protein concentration on SDS-PAGE or at concentrations lower than the dilution of the antibody in immunoblotting. The densities of the immunochemically stained bands were measured using a scanning densitometer. The density ratio (the 105-kDa protein/HSP90) was 0.5. Both were detected only in the cytosol fraction but not in the microsormal, mitochondrial but nuclear fractions (data not shown).

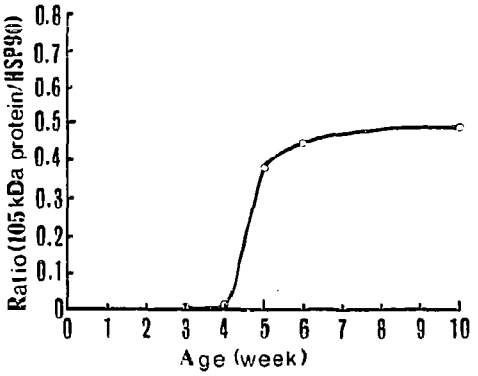

Fig. 3. Growth curve of the 105-kDa protein for each age of rat testis. The densities of the immunochemically stained bands (Fig. 2B) were measured using a Hoffer scanning densitometer, and the density ratio of the 105-kDa protein and HSP90 for each age was plotted.

The appearance of the 105-kDa protein and HSP90 in rat testis was studied. The testis cytosols from 3-, 4-, 5-, 6-, and 10-week-old rats were electrophoresed on SDS-PAGE and immunoblotted using anti-HSP90 antibody (Fig. 2). HSP90 could be detected from 3-week-old rat testes. The 105-kDa protein appeared at least two weeks later than HSP90 and was observed from the 5-week-old sample. The ratio of the 105-kDa protein to HSP90 in each age was measured using a scanning den-

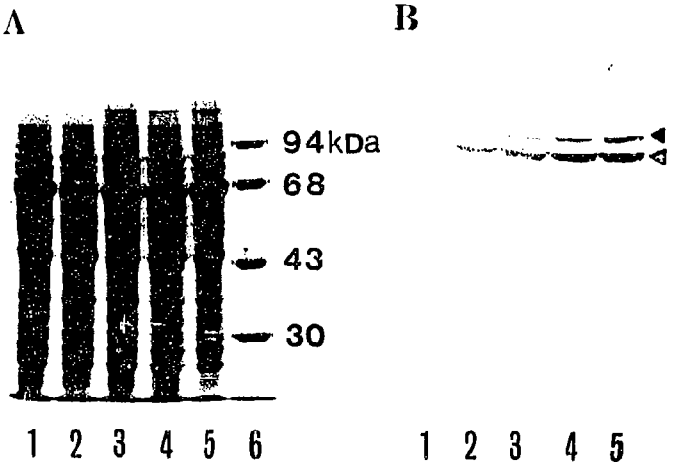

Fig. 2. Expression time of the 105-kDa protein and HSP90 in rat testis. Each age sample of rat testis was homogenized with 10 mM Tris-HCl, 0.125 M NaCl (pH 7.4). After centrifugation at 18 000 × g for 20 min, the cytosols were analyzed by SDS-PAGE (9% gel, 100 μg protein per lane). The gels were stained with Coomassie brilliant blue (panel A), or immunoblotted with anti-HSP90 antibody (panel B). Lane 1, 3-week-old; lane 2, 4-week-old; lane 3, 5-week-old; lane 4, 6-week-old; lane 5, 10-week-old; lane 6, standard marker proteins. In panel B, the closed and open triangles indicate the 105-kDa protein and HSP90, respectively.

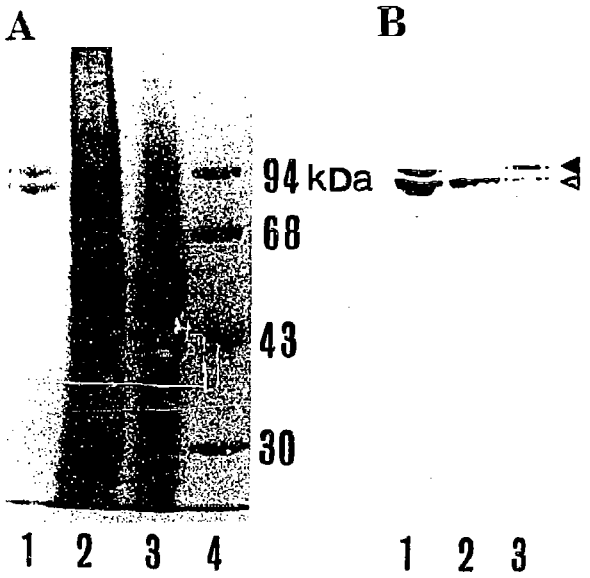

Fig. 4. Immunological detection of the 105-kDa protein and HSP90 in LC540 g cells and spermatozoa. LC-540 cell cytosol and rat spermatozoan cytosol, as in section 2.2 and 2.3, were electrophoresed on 9% SDS-polyacrylamide gel (50 μg per lane) and subsequently immunoblotted. (A) The gel was stained with Coomassie brilliant blue. (B) The PVDF membrane after electrophoretic transfer was stained using anti-HSP90 antibody. Lane 1, rat testis crude extract; lane 2, LC-540 cell cytosol; lane 3, spermatozoan cytosol; lane 4, standard marker proteins. The closed and open triangles indicate the 105-kDa protein and HSP90, respectively.

sitometer (Fig. 3). The value reached the maximum roughly one week after the appearance of the 105-kDa protein.

The cytosol of LC-540 cells (established from rat Leydig cell tumor) and rat spermatozoan cytosol were electrophoresed on SDS-polyacrylamide gels. The gel was stained with Coomassie brilliant blue (Fig. 4A) or immunoblotted using anti-HSP90 antibody (Fig. 4B). The 105-kDa protein was detected only in the spermatozoan cytosol but not in the LC-540 cells cytosol, in contrast to HSP90 which was detected in both cytosols.

4. DISCUSSION

The present results have confirmed that the 105-kDa protein is one of the sperm-specific proteins. Our previous studies showed that this protein is one of the HSP90 family relative to primary structure and immuno-cross reactivity [3].

During the development of rat, this protein appeared at approximately the age of 5 weeks, coinciding with the appearance of spermatozoa. These results are similar to phosphoglycerate kinase-2 (PGK-2), a sperm-specific protein which appears at the age of 30 days [7,8]. The ratio of the 105-kDa protein to SP90 reached the maximum almost immediately. This means that both proteins increase in parallel. The 105-kDa protein is principal in spermatozoa in place of HSP90.

Lydig cells secrete androgens which are essential for spermatogenesis. LC540 cells originate in Leydig cells and maintain the androgen-secretion activity. The 105-kDa protein was not observed in this cell line at all. These results suggest that the 105-kDa protein bears some sperm-specific functions.

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