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COMMON ANTIGENICITY OF MOUSE 42°C-SPECIFIC HEAT-SHOCK PROTEIN WITH MOUSE HSP 105

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SUMMARY Mammalian cells incubated at 42°C synthesize a specific heat-shock protein at 42°C (42°C-hsp) that is not induced by heat-shock at 45°C or by other stresses that induce major heat shock proteins (Hatayama et al. (1986) Biochem. Biophys. Res. Commun. 137, 957-963). Antibody raised against a heat-shock protein with molecular weight of 105,000 (hsp 105) purified from mouse FM 3A cells cross-reacted to the 42°C-hsp of the same cells. The antibody reacted only weakly to hsp 105 and 42°C-hsp of human HeLa cells. These results suggested that hsp 105 and 42°C-hsp have the same antigenic determinant, and that 42°C-hsp may have a structure similar to that of hsp 105.

Heat-shock proteins, the synthesis of which is accelerated by heat or other environmental stresses, are found in vast numbers of species (1, 2). The molecular structures of heat shock proteins are conserved well throughout evolution (3, 4). Major heat-shock proteins with molecular weights of 70,000, 73,000, 85,000, and 105,000 (hsp 70, hsp 73, hsp 85, and hsp 105, respectively) are found in many different mammalian cells (5).

One particular heat shock protein is synthesized in mammalian cells incubated at 42°C (6, 7). This 42°C-specific heat-shock protein (42°C-hsp) is absent in the cells grown at 37°C or in the cells stressed by heat shock at 45°C or by chemical agents

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Abbreviations: hsp, heat-shock protein; SDS, sodium dodecyl sulfate; PBS, phosphate-buffered saline.

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that induce major heat shock proteins. The 42°C-hsp is induced in human HeLa cells as well as in mouse FM 3A cells (7).

The molecular weight of 42°C-hsp is 90,000, and the isoelectric point is 5.6. By two-dimensional gel electrophoresis, the 42°C-hsp has been separated into two isomers with slightly different isoelectric points. By cell fractionation study, the 42°C-hsp is found to be in the cytosol fraction only, and not in the nuclear or other particulate fractions (7).

In this paper, we show that antibody raised against the hsp 105 of mouse FM 3A cells cross-reacted to 42°C-hsp of FM 3A cells.

MATERIALS AND METHODS

Cell culture and heat treatment of the cells

FM 3A cells or HeLa cells were grown in Eagle's minimum essential medium supplemented with 10% calf serum as a suspension culture or as a monolayer culture, respectively. Exponentially growing cells were heat-shocked at 42°C in a CO_2 incubator for various times.

Two-dimensional gel electrophoresis

Proteins (0.5 mg) or [^{35}S]methionine-labeled proteins (200,000-1,000,000 cpm) were treated with RNase A and analyzed by two-dimensional gel electrophoresis (8). For the first dimension, the isoelectric focusing gel containing 1.6% pH 5-7 ampholytes (Fig. 1) or pH 6-8 ampholytes (Fig. 2), and 0.4% pH 3.5-10 ampholytes were used. For the second dimension, 10% polyacrylamide SDS slab gels were used. The gels for the [^{35}S]methionine-labeled proteins were processed with Amplify (Amersham) and fluorographed at -80°C.

Production of anti-mouse hsp 105 antibody

Hsp 105 was purified from FM 3A cells incubated at 42°C for 6 hours. The cells (1.5×10^{10}) were homogenized in hypotonic buffer (20 mM Tris-acetate pH 7.6 and 1 mM phenylmethanesulfonyl fluoride). The cell extract was obtained by a centrifugation at 105,000 x g for 1 hour at 4°C and chromatographed first on a DEAE-Sepharose CL 6B column and then on a hydroxylapatite column. Hsp 105 was further purified by two-dimensional gel electrophoresis and the spot of hsp 105 stained with Coomassie blue was cut out from the gel and stored at -20°C until use.

For immunization, 15 pieces of the gel containing hsp 105 (per rabbit) were neutralized and homogenized in PBS with a Teflon-glass homogenizer, emulsified with Freund's complete adjuvant , and injected subcutaneously into a rabbit (New Zealand white or Japanese white) at several places in the back and foot pads. Borditella pertussis vaccine (1 ml; Chiba Kessei, Japan) was injected at the same time subcutaneously in other points on the back (9). For booster injections, homogenates from 10 pieces of gel containing hsp 105 were emulsified with Freund's incomplete adjuvant and injected subcutaneously at 4 weeks and 8 weeks after the first injection. The antiserum obtained 8 days after the second booster injection was used in this study. For the immunoprecipitation study, the rabbit IgG fraction was obtained from the serum by precipitation with ammonium sulfate at 33-50% saturation.

Western blotting and immunological detection of hsp 105

Proteins separated on the one- or two-dimensional gels were blotted onto a nylon membrane (Biodyne) by electrotransfer (first 50 mA for 1 h, and then 110 mA overnight) in 5 mM borate-NaOH buffer (pH 9.0). The membrane was incubated first with 20% fetal calf serum in PBS, and then with anti-mouse hsp 105 antiserum or pre-immune serum (each diluted 50-fold). After washing with 0.5% Tween 80-PBS and with PBS, the membrane was incubated with anti-rabbit IgG antibody conjugated with peroxidase (Cappel Laboratories; diluted 1,000-fold). After washing the membrane with 0.5% Tween 80-PBS and washing with PBS, the immune complexes were made visible by incubation of the membrane in 50 mM acetate buffer (pH 5.0) containing 0.04% 3-amino-9-ethylcarbazole and 0.015% H_2O_2.

Immunoprecipitation

FM 3A cells (3-5 x 10^5/ml) or HeLa cells (5-7 x 10^5/cm^2) were labeled with [^{35}S]methionine in methionine-deficient medium supplemented with 10% calf serum. The cells were washed twice with cold PBS and homogenized in hypotonic buffer. After centrifugation at 12,000 x g for 20 min at 4°C, portions of the extracts (about 500,000 cpm) were incubated with pre-immune or immune IgG (1-2 mg) in PBS at 4°C for 2 hours, and then 100 µl of 50% protein A-Sepharose CL-4B (Sigma) in PBS was added. The mixtures were incubated at 4°C overnight and centrifuged at 12,000 x g for 20 min at 4°C. The precipitates were washed several times with cold PBS. The proteins bound to protein A-Sepharose were released from the precipitates by the addition of 100 µl of SDS sample buffer. The unbound proteins in the first supernatant and the bound proteins released into the SDS sample buffer were analyzed by two-dimensional gel electrophoresis.

RESULTS

The immunoreactivity of anti-mouse hsp 105 serum was analyzed by Western blotting of the proteins of the FM 3A and HeLa cells separated by one-dimensional gel electrophoresis. The antiserum reacted strongly with the protein with the molecular weight of 105,000 from FM 3A cells. The amount of the reactive protein of FM 3A cells increased when these cells were exposed to heat shock, so the protein seemed to be hsp 105. As anti-mouse hsp 105 serum reacted only weakly to the corresponding protein of HeLa cells, there seemed to be species specificity in the structure of hsp 105.

To analyze the immunoreactivity of the anti-mouse hsp 105 serum in more detail, proteins of FM 3A cells were separated by two-dimensional gel electrophoresis (Fig. 1). The antiserum reacted to the protein, with or without heat shock, that migrated to the position of hsp 105 on the two-dimensional gels. The antiserum also reacted to the protein that migrated to the position of 42°C-hsp in the cell extract from FM 3A cells heated at 42°C, but not in the extracts from cells incubated at 37°C or heated at 45°C. Some non-specific reactions were weakly seen at the

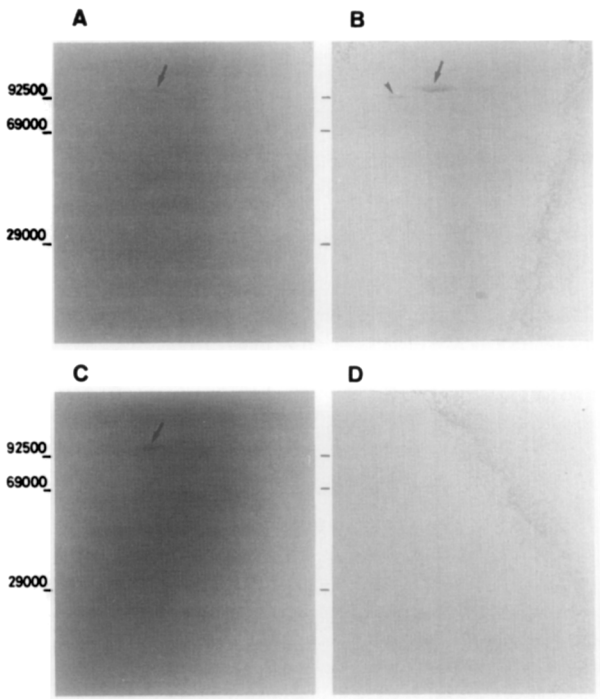

Figure 1 Immunoreactivity of anti-mouse hsp 105 serum to mouse hsp 105 and 42°C-hsp.
FM 3A cells were incubated at 37°C (A), at 42°C for 6 h (B, D), or at 37°C for 6 h after heat shock at 45°C for 10 min (C). Proteins (500 μg) from the cell extracts were separated by two-dimensional gel electrophoresis and transferred to nylon membranes. The membranes were incubated with antiserum against mouse hsp 105 (A-C) or with pre-immune serum (D), and the immune complexes were made visible as described in Materials and Methods. Arrows and arrowheads indicate the position of hsp 105 and 42°C-hsp, respectively. The molecular weights of the marker proteins are indicated at the left side of the membrane.

regions of molecular weights of 75,000-90,000, where distinct spots stained with Coomassie blue were not observed.

To confirm the cross-reactivity of the anti-mouse hsp 105 antibody to 42°C-hsp, [^{35}S]methionine-labeled proteins of FM 3A cells were immunoprecipitated by the antiserum IgG and the immune complexes were precipitated with protein A-Sepharose. The bound and unbound proteins were analyzed by two-dimensional gel electrophoresis. Hsp 105 and 42°C-hsp were specifically absorbed to anti-mouse hsp 105 IgG and then released from the precipitates into the SDS sample buffer (Fig. 2, D). Some other proteins were also released from the precipitates, but these proteins seemed to bind non-specifically to rabbit IgG and protein A-Sepharose (Fig. 2, E, F). That anti-hsp 105 IgG reacted not only to hsp 105 but also to 42°C-hsp suggested that hsp 105 and 42°C-hsp had the same

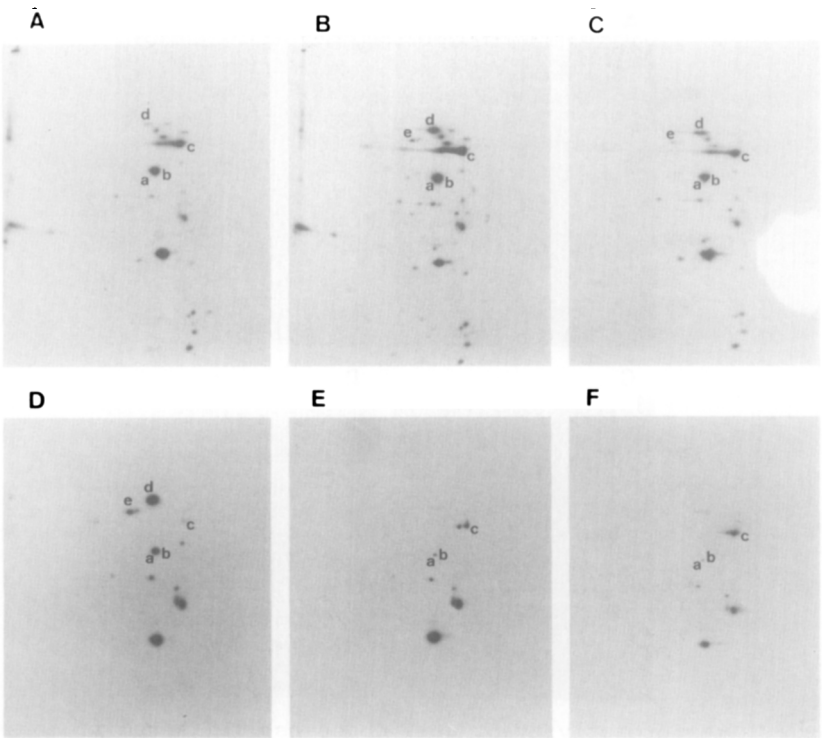

Figure 2 Immunoprecipitation of [^{35}S]methionine-labeled pro ns of FM 3A cells with anti-mouse hsp 105 IgG.
FM 3A cells were labeled with [^{35}S]methionine at 42°C for 3 h. The proteins from the cell extracts were incubated with anti-mouse hsp 105 IgG (A, D), or with pre-immune IgG (B, E) or without IgG (C, F), and further incubated with protein A-Sepharose to absorb the immune complexes. Proteins not bound to the protein A-Sepharose (A-C) and proteins released from the protein A-Sepharose (D-F ; obtained from three times the amount of sample used for A-C, respectively) were analyzed by two-dimensional gel electrophoresis. a, b, c, d, and e indicate hsp 70, hsp 73, hsp 85, hsp 105, and 42°C-hsp, respectively.

antigenic determinant and probably share a structure that is the same in part. When [^{35}S]methionine-labeled proteins of HeLa cells were immunoprecipitated with anti-mouse hsp 105 IgG and protein A-Sepharose, small amounts of both hsp 105 and 42°C-hsp were absorbed to the antibody (data not shown).

DISCUSSION

We showed that anti-mouse hsp 105 antibody reacted not only to hsp 105 but also to the 42°C-hsp of mouse FM 3A cells. This indicated that hsp 105 and 42°C-hsp have common antigenicity and probably have a similar structure.

Hsp 70, hsp 85, and hsp 105 are generally observed in mammalian cells. The first two are well conserved in divergent species from bacteria to humans, but hsp 105 has been found only in mammalian cells (5). In mouse embryonic carcinoma cells, hsp 105 is inducible only after differentiation of the cells, whereas hsp 70 is inducible at undifferentiated as well as differentiated stages of the cells (10). Thus, hsp 105 may have some specific functions in differentiated mammalian cells.

An immunocytochemical study done with mouse kidney cells has shown that hsp 105 is located in the cytoplasm and perinuclear regions of unstressed cells, and some move to nucleus or nucleolus during heat shock (11). Other immunocytochemical studies done with Chinese hamster ovary cells and mouse embryo fibroblast cells showed that hsp 105 is located in the nucleolus of the cells even at normal conditions (12, 13). The hsp 105 at nucleolus is released by RNase treatment of the cells, suggesting that hsp 105 is associated with RNA components in the cells (12). On the contrary, our cell fractionation study with FM 3A or HeLa cells reveals that hsp 105 and 42°C-hsp are only in the cytosol fraction, not in the nuclear or other cytoplasmic particulate fractions in these cells (7).

There is much evidence of a correlation between the intracellular accumulation of heat-shock proteins and the thermotolerance of the cells (14, 15). Thermotolerance develops in cells heat-shocked at 45°C as well as at 42°C (16), so 42°C-hsp seems not to have an essential role for the development of thermotolerance in the cells.

42°C-hsp is a particular heat-shock protein that is found only in cells incubated at 42°C; it is completely absent in cells grown at 37°C or in cells stressed by heat shock at 45°C or by chemical agents that induce the major heat-shock proteins. So, there may be a specific function and induction mechanism for the 42°C-hsp that are different from those of other heat-shock proteins. To elucidate the specific function and induction mechanism of 42°C-hsp in detail, we are now investigating the gene structure of 42°C-hsp.

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