

Purification and identification of 35KDa heat stable protein

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(Received: April 12, 2008; Accepted: June 04, 2008)

ABSTRACT

A heat stable protein (hsp) has been purified to homogeneity from sheep liver extract by heating to 95°C followed by anion-exchange chromatography on DEAE-cellulose (DE-52). The purified protein showed a single band when examined by gel electrophoresis. The molecular weight determined by SDSPage was found to be approximately 35KDa. The presence of this protein was also demonstrated in whole cell homogenate, heat stable and nuclear fraction.

Key words: Hsp, DEAE, SDSPage.

Heat shock proteins are constitutively expressed making up to 5-10% of the total protein content under normal growth conditions, but their intracellular concentration can increase up to several fold by insults that induce protein unfolding, misfolding or aggregation, and a flux of newly synthesized non-native proteins. The term heat shock protein is something of a misnomer since in addition to raised temperature, the expression of these proteins is also induced upon exposure to oxidative stress, ultraviolet irradiation, chemical substances and viral infection and in nutritional deficiency (Nover 1991, Welch 1993, Fincato 1991, chauchanel 1994).

Methodology

Preparation of crude liver extract

Frozen sheep liver was cut into small pieces then homogenized in lysis buffer containing (10mM Tris acetate pH=7.5, 10mM NaCl, 1mMEDTA, 1mMPMSF) using a hand held homogenizer. The homogenate was centrifuged at 700g for 30 minutes. Pellet was discarded and the supernatant recentrifuged at 700g for 30 minutes. The supernatant were saved as total cytosolic protein extract.

Preparation of boiled extract

Crude extract prepared as was incubated at 95°C water bath for 7-10 min with constant stirring and cooled on ice. The precipitated protein was discarded following centrifugation and the remaining supernatant was saved as heat stable fraction.

Deae-cellulose chromatography

Boiled extract was applied separately on to a DEAE-Cellulose column {4cm×2mm} equilibrated with buffer A (20mM Tris acetate pH=7.6, 20mM NaCl, 0.1mMEDTA). After washing the column with buffer A until the absorbance of the eluate decreased to less than 0.025 at 280nm. Mixture of proteins bound as a yellow zone at top of column was eluted with a linear 40mM-500mM NaCl gradient in buffer A at a flow rate of 25ml/hour. Gradient volume used was 5 times the bed volume of mini column [4ml]. Fractions of 0.5ml of the eluate collected. 50 fractions were collected and all fractions visualized on sds/page.

Nuclear protein extraction

Nuclear protein extract were prepared according to method of Zhu *et. al* (2001). To the nuclear pellets, ice cold high salt buffer containing [20mM Hepes, 25% Glycerol, 0.42M NaCl 0.2mM EDTA, 1.5mM MgCl₂, 0.5mM PMSF) was added and mixed nuclei were incubated for 15 mins and centrifuged at maximum speed for 1 min. Supernatant were saved as nuclear protein extract.

Protein estimation

Protein concentrations at each step of the purification was determined by Bradford method using bovine serum albumin as standard.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SDSPage was carried out in 12%

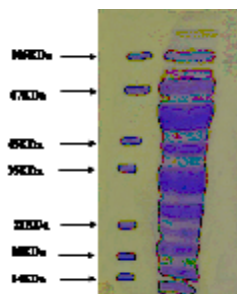


Fig. 1: Show SDS page pattern of liver homogenate (volume loaded 30 μ l)

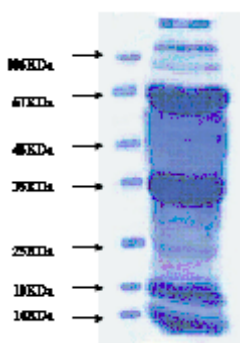


Fig. 2: Heat stable proteins from liver homogenate (volume loaded 30 μ l)

separating gel with a 5% stacking gel according to lamilli. The proteins were visualized by staining with 0.1% coomassie brilliant blue R250.

Western blotting

Different protein samples were separated on 10% polyacrylamide gels and then transferred to a PVDF membrane (Himedia) for one hour at 75V in a transfer buffer containing (24mM Tris base, 0.2M Glycine 0.1% SDS, and 20% Methanol). Non specific binding sites on the membrane was blocked using 3% bovine serum albumin in tris buffered saline pH-7.4 for overnight at 37C. Membrane was

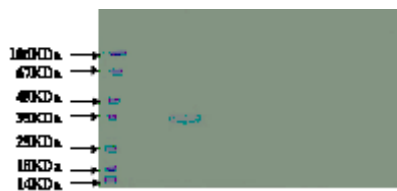


Fig. 3: SDS page of purified 3540 KDa protein (volume loaded 30 μ l)

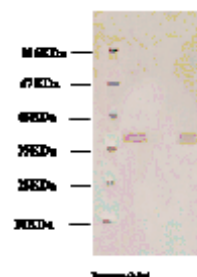


Fig. 4: Left lane q, molecular weight marker (volume loaded 10 μ l) lane 2, crude liver extract lane 3, heat stable fraction lane 4, nuclear extract (volume loaded in each lane 20 μ l)

incubated with primary antibody (1 μ g/ml) After washing, antibody binding was visualized by alkaline phosphatase conjugated secondary antibody (1 μ g of ALP) with 5-Bromo-4 chloro-3 nitroblue tetrazolium as substrate. Color development was stopped by 20mM EDTA.

Western analysis of 35 kda protein

In purification procedure protein of molecular weight (35-40KDa) was partially purified. To localize the presence of this protein in crude liver extract, heat stable fraction, and nuclear extract. All the three fractions were saperated by SDSpage and western blotted using anti hsp 40 antibody. A band was highlighted in whole cell homogenate and nuclear extract above 35KDa.

REFERENCES

1. Nover L., Heat shock response. Boca Raton; CRC Press (1991).
2. Welch, W.J., and Mizzen, L.A. Characterization of the thermotolerant cells.II. Effects on the intracellular distribution of heat shock protein 70, intermediate filaments and small nuclear ribonucleoprotein complexes. *J. Cell Biol.* **106**: 1117-1130 (1988).