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]. 50 fractions were collected and all fractions visualized on SDSpage.

**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 0.2mMEDTA, 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: SDS page pattern of liver homogenate (volume loaded 30µl)

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

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

Fig. 4: Left lane q, molecular weight marker (volume loaded 10µl) lane 2, crude liver extract lane 3, heat stable fraction lane 4, nuclear extract (volume loaded in each lane 20µl) incubated with primary antibody (1µ g/ml) After washing, antibody binding was visualized by alkaline phosphatase conjugated secondary antibody (1µg of ALP) with 5-Bromo-4 chloro-3 nitroblue tetrazolium as substrate. Color development was stopped by 20mM EDTA.

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

REFERENCES