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Purification of hsc70 from liver tissue

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ABSTRACT

A 70KDa protein has been purified by anion exchange chromatography on (DE-52).Molecular weight of protein was also determined by employing 10% SDS-page with phosphorylase b(Mr 92,500) BSA (Mr 68000) ovalbumin(Mr 43,000) chymotrysinogen(Mr 27,000) and Myoglobin(Mr17200) as the marker proteins.After electrophoresis gel was stained with 0.25% CBB for 4 hour and were then destained.

Key words: SDS DEAE, CBB.

Heat shock proteins (Hsps) were discovered in 1962 when Ferruccio Rittosa and his coworkers noted that temperature shock produced odd-puffing patterns and an unusual profile of gene expression in the polytene chromosomes of salivary glands in Drosophilla melanogaster larva. These constituvively expressed proteins play a fundamental role in maintaining the stability of other proteins within cells.In mammals these comprise hsps 100,90,70,60,and sHsps (coffer *et al* 1985).These protein take part in the assembly stabilization,folding and translocation of oligomeric proteins.(Hightower 1991, Gething 1992).

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^{0}C water bath for 7-10 minutes with constant stirring and cooled on ice. The precipitated protein was discarded following centrifugation and the remaining supernatant was saved as heat stable fraction.

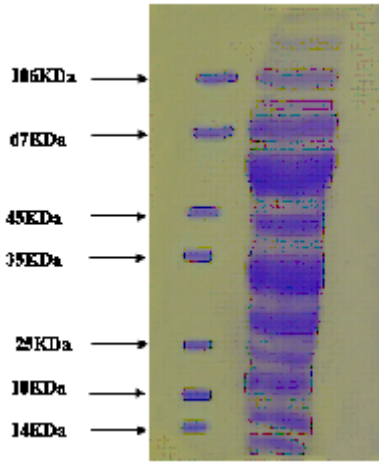

Fig. 1: Show SDS page pattern of liver homogenate (volume loaded 30µl)

Deae-cellulose choromatography boiling stable was applied to 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.

Fig. 2: 50% pellet SDS page of purified 70KDa protein (Volume loaded 20µl)

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

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