Purification of Human Heat Shock Protein (Hsp90) Using a Bio-Scale CHT2-I Column

Introduction

The 90 kDa heat shock protein (Hsp90) is one of the most abundant proteins in the cytosol of eukaryotic cells. *In vivo* it has been shown to interact with a variety of cellular target proteins that participate in signal transduction pathways such as steroid receptors and certain kinases. *In vitro*, Hsp90 can act as a molecular chaperone preventing the aggregation of unfolded substrate proteins. For *in vitro* experiments, highly purified protein is an essential prerequisite. This application note describes an improved purification protocol of human Hsp90 using the Bio-Scale CHT2-I ceramic hydroxyapatite column (catalog # 751-0021).

Hsp90 is very sensitive to degradation by proteases, thus a rapid purification protocol that delivers high protein purity is required. The traditional purification scheme involved capture on a cellulose anion exchange support (DE52) followed by a crystalline hydroxyapatite step (HTP) and gel filtration. The development of the more mechanically stable ceramic hydroxyapatite allowed the use of higher flow rates (2 ml/min vs 0.5 ml/min) than could be used with HTP crystalline hydroxyapatite. Use of the Bio-Scale CHT2-I, 10 µm ceramic hydroxyapatite column provided increased separation efficiencies and faster separations which resulted in higher protein recoveries due to reduced proteolytic degradation.

Purification of Hsp90

Human Hsp90 was expressed in baculovirus infected cells and was purified using three chromatographic steps. The cells were homogenized and the soluble fraction was loaded onto an anion exchange column. The Hsp90 containing fractions were combined and dialyzed against 20 mM potassium phosphate, pH 6.8, and were injected onto a 2 ml Bio-Scale CHT2-I column. A 10 column volume linear gradient from 20–400 mM potassium phosphate, pH 6.8, was run at 2.0 ml/min (Figure 1). Twenty 1 ml fractions were collected and were analyzed by SDS-PAGE (Figure 2). Fractions 11–15 were pooled and run on a high resolution gel filtration column. Recovered Hsp90 protein was >99% pure according to SDS-PAGE.

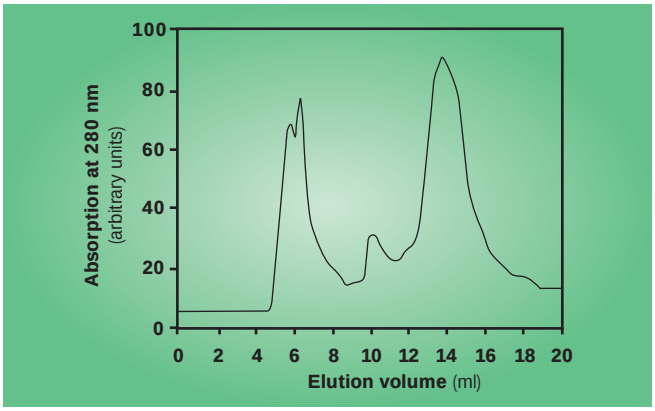

Fig. 1. Elution profile of pooled anion exchange fractions of human Hsp90 from a Bio-Scale CHT2-I column. Buffer A: 20 mM potassium phosphate, pH 6.8. Buffer B: 400 mM potassium phosphate, pH 6.8. Gradient: 0–100% B in 20 ml. Flow rate: 2.0 ml/min. Detection: 280 nm.

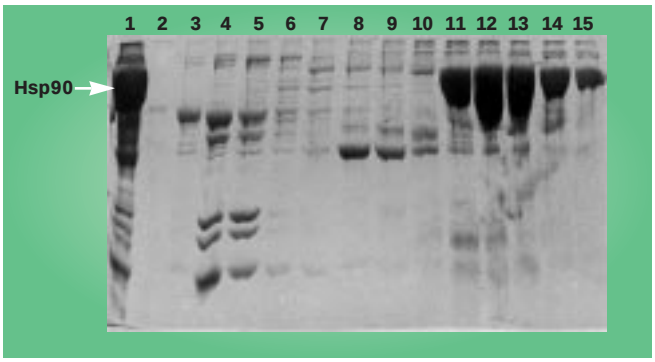

Fig. 2. SDS-PAGE of human Hsp90 on 10% acrylamide gel. Lane 1: Anion exchange fraction loaded onto Bio-Scale CHT2-I column. Lane 2: Flow through of Bio-Scale CHT2-I column. Lanes 3–15: fractions 5–17. Hsp90 is a 90kDa protein.

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