Cortactin is an actin-binding protein that has been shown to be involved in cellular migration and metastasis in cancer. Bacterially expressed and purified cortactin protein is often used in vitro assays to examine cortactin’s role in facilitating motility phenotypes. To elucidate the forms of cortactin produced from bacterially expressed and purified cortactin, we used a two-step purification system including affinity purification and anion exchange chromatography. After analysis with non-denaturing polyacrylamide gel electrophoresis, we found cortactin protein from different anion chromatography elution fractions did not separate to similar locations on the gel across all fractions. We hypothesize that the variations in the bands are a result of different folding patterns of cortactin protein in what was once thought of as a homogenous protein pool. When testing the role of cortactin in mediating cell migration, these folding differences may have significant effects on the results of functional assays such as actin polymerization or sedimentation.

**Hypothesis**

Cortactin is upregulated in a number of metastatic cancers, and is known to be involved in cell migration. After finding the different binding patterns of cortactin among the different fractions of KCl elutions, we concluded that our hypothesis was possible. There seems to be definite structural differences between the cortactin proteins in the different elutions. The significance of the differences remains to be seen through functional assays of their actin assembly activity.

**Background**

- Cortactin is a protein that binds to actin networks within the cell and acts with the Arp 2/3 actin nucleator complex and several other proteins to promote actin remodeling. It has been found that cortactin phosphorylated with different kinases actually affect cell migration in distinct ways, for example serine phosphorylated cortactin affects actin assembly and tyrosine phosphorylated forms affect focal adhesion turnover.
- Various states of phosphorylation may cause different forms of cortactin that perform different functions in the cell possibly other than cell migration. These different structures of cortactin with different phosphorylation states might exist in the cell simultaneously but there structures and possible functions remain unknown. It was not until relatively recently that anyone was able to demonstrate significant actin assembly by cortactin due to the fractionation of the protein through an anion exchange column. This is important because it suggests that there may be research groups experimenting with cortactin in different folded forms. This study could lead to more precise experimentation with cortactin.

**Results & Discussion**

**Figure 1: Experimental Model**

**Figure 2: Purification**

A. Cortactin was first purified by Ni²⁺ affinity chromatography followed by anion exchange chromatography. Coomassie stain of cortactin protein fractions eluted with increasing concentrations of KCl (0.4 M to 0.5 M), separated by SDS-PAGE

B. Western blot of of cortactin protein fractions eluted with increasing concentrations of KCl (0.4 M to 0.5 M) using 4F11 monoclonal anti-cortactin antibody.

**Figure 3: Native and SS Gels**

A. Cortactin is stained by both a silver stain (A) and a KE-20 polyclonal antibodies specific for cortactin (B).

**Future Directions**

- **Future question:** Do the different fractions of cortactin have different functional abilities?
- **Possibilities:**
  - Use each cortactin fraction in an actin polymerization assay
  - Analyze bands by mass spectroscopy to confirm identical sequences
  - Analyze post-translational modification patterns across fractions

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**References**