Purification of cortactin using anion exchange chromatography

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ABSTRACT

Cortactin is an actin-binding protein that has been shown to be involved in cellular migration and metastases in cancer. Bacterially expressed and purified cortactin protein is often used in in vitro assays to examine cortactin's role in promoting cell migration via actin remodeling. Cortactin has a theoretical molecular weight of 60 kDa, however using SDS-PAGE analysis the protein runs as two bands of molecular weights 80 kDa and 85 kDa suggesting that cortactin has an unusual protein folding pattern. Our current lack of understanding of cortactin structure limits our ability to determine the role of cortactin in facilitating motility phenotypes. To elucidate the forms of cortactin produced from bacterially expressed and purified cortactin protein, 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.

BACKGROUND

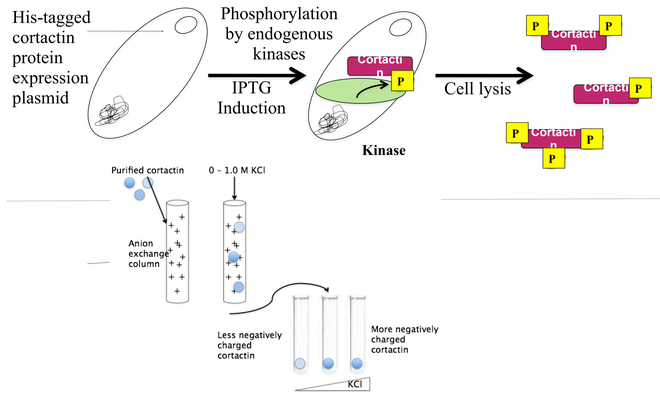
- Cortactin is a protein that binds to actin networks within the cell and acts with the Arp 2/3 actin nucleator complex and several other proteins to promote actin remodeling[2].
- There are 27 known phosphorylation sites on cortactin, some of whose roles are known[3].
  - Phosphorylation of tyr^{421}, tyr^{466} and tyr^{482} increase actin polymerization and invadapodia in cells via the ERK and SRC kinase systems[2].
- *E. coli* has a number of kinases allowing it to phosphorylate proteins at Ser/Thr/Tyr.
- 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 a 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.

HYPOTHESIS

Cortactin is upregulated in a number of metastatic cancers, and is phosphorylated on 27 separate amino acids. The protein is acquired using recombinant DNA techniques, and the possibility of post translational modifications of cortactin in *E. coli* has been ignored to this point.

We now hypothesize that cortactin protein exists in a number of folded forms that may have different functional properties, and these forms can be separated by anion exchange

FIGURE 1: EXPERIMENTAL MODEL



Post Translational Modifications

E. Coli contain many endogenous kinases, some of which are homologues to eukaryotic kinases.
These endogenous kinases phosphorylate cortactin during induction and expression of the his-tagged cortactin protein expression vector.
Protein purified from this system is post-translationally modified and are not appropriate for use in *in vitro* assays without phosphatase treatment.

Purification and fractionation of Cortactin:

His-cortactin proteins were prepared with recombinant DNA technology using E. coli. The culture was prepared with a 1000x dilution of Ampicillin (Amp) and Chloramphenicol (Cam). IPTG was added and the culture was incubated for four hours and cells were centrifuged at 3000 r.p.m. in a floor centrifuge. The cells were resuspended using lysis buffer and then were spun at 14,000 r.p.m. in the floor centrifuge. The supernatant was removed and passed through a nickel-nitrilotriacetic acid (Ni-NTA) metal affinity beads at a flow rate of 1 mL per minute. The column was washed with lysis buffer and the His-cortactin with eluted with elution buffer. Eluted fractions were pooled together and run through a Mono-Q column. The protein was eluted with elution buffer against a 0-1 M KCl gradient at a flow rate of 1 mL per minute.

FIGURE 2: PURIFICATION

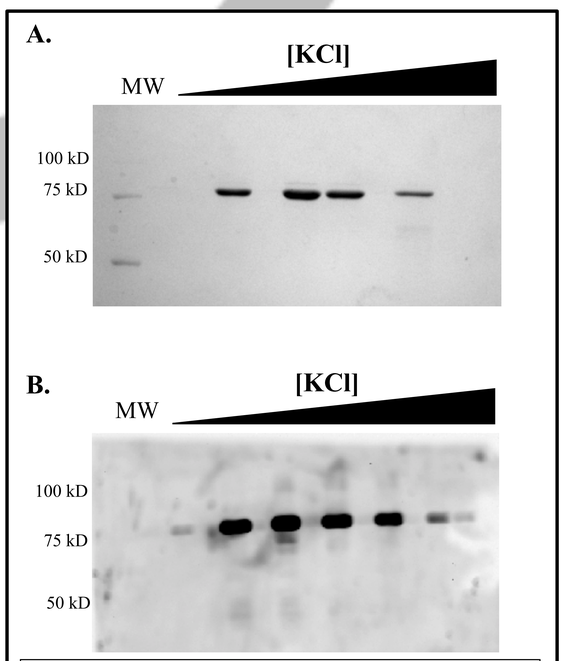


Figure 2: Purification of cortactin from E. coli

A.Cortactin was first purified by Ni^{2+} 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

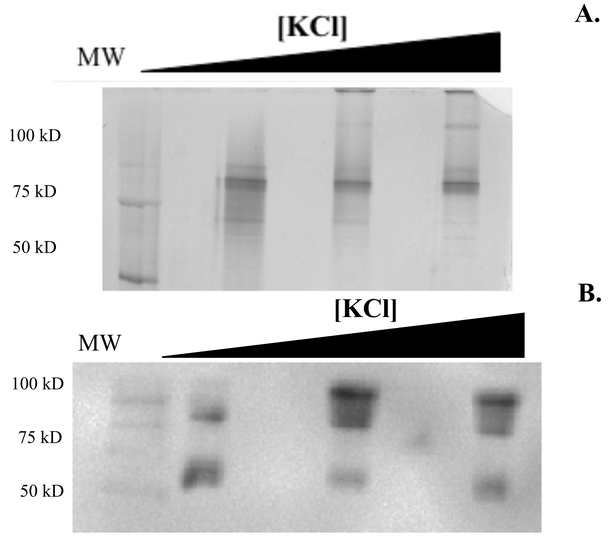


Figure 3: Cortactin is stained by both a silver stain (A) and a KE-20 polyclonal antibodies specific for cortactin (B).

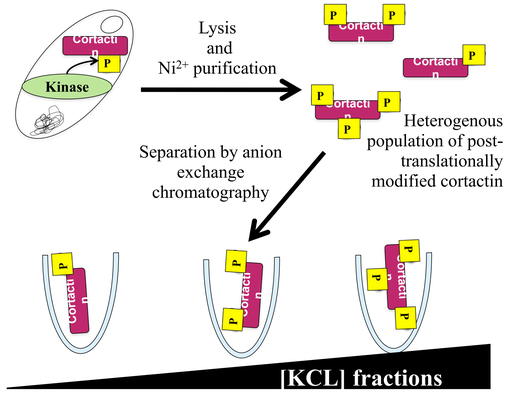
A.The products of a non denaturing electrophoresis gel visualized using a silver stain. Due to the high sensitivity of the stain and the native form of cortactin running through the gel, banding lanes were detected in areas other than the known molecular weight of cortactin.
B.The polyclonal antibody for cortactin was used to stain a western blot which was run on a non denaturing electrophoresis gel. Once again multiple banding patterns were visualized on this blot outside of the natural cortactin banding patterns.

RESULTS & DISCUSSION

After finding the different banding 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



FUTURE DIRECTIONS

- Future question: Do the different fractions of cortactin have different functional abilities?
- Possibilities:
- Use each cortactin fractions 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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