Determining the Foldedness of IkBα P261F and IkBα E282W

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Background

Protein Expression and Purification

Data and Results

Background

IκBα Interaction with NFkB

Transformation

Incubate Cells with DNA
1 μg DNA was added to 50 μL of E. coli (BL21) cells and incubated on ice for 20 min

Heat Shock
Cells were incubated at 42 °C for 10 min and then incubated at 37 °C for 2 min

Transfer Starter Culture
Transfer 500 μL of competent cells to 1 mL of LB media and shake at 37 °C

Shake and Grow
Grow cells until OD600 ~ 0.4

Pre-Induction
Spin down and re-suspend pellet in 50 μL of water and then add 1 μL of IPTG for 16 h

Induction
Put flask in shaker at 18 °C overnight.

Conclusions and Future Directions:

• IκBα WT was successfully expressed and purified.
• IκBα P261F and E282W mutant plasmids were transformed into BL21 (DE3) E. coli cells.
• The expression of the IκBα P261F and E282W mutants was not optimal under traditional IκBα expression conditions.
• I plan to optimize the expression of IκBα P261F and E282W mutants.
• I will analyze the foldedness of the IκBα P261F and E282W mutants by urea denaturation circular dichroism experiments.
• Once the mutants are purified, I will test the binding affinities of the mutants to NFkB, and I will test the abilities of the mutants to “strip” NFkB from DNA.

References


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Above: Nuclear factor-κB (NFκB) and its inhibitor, IκBα. PDB 1KRN.

Above: Circular dichroism (CD) spectrum to determine the foldedness of a protein. (a) non-structured protein and (b) α-helical protein.1

Above: Fraction unfolded in equilibrium denaturation experiments with IκBα WT (circles) monitored by CD signal at 225 nm (filled circles) and fluorescence (red circles).

Above: Urea denaturation curves followed by the CD signal at 225 nm of IκBα Y254L/T257A (filled circles) and fluorescence (red circles). 15

Above: Transformation of BL21 (DE3) E. coli with pET11a/IκBα P261F plasmid on an LB agar plate that was incubated at 37 °C overnight.

Above: S75 size exclusion chromatograms for IκBα WT (fraction 17) and IκBα E282W (fraction 14).

Left: The structure of IκBα shows that two site mutants which are at P261 and E282. Mutant P261 is in Ankyin Repeat 6 and E282 is at the end of Ankyin Repeat 6.

Above: Western blot analysis of IκBα WT, wild-type, Y254L/T257A, and P261F, E282W mutants.

Above: Western blot analysis of IκBα WT, wild-type, Y254L/T257A, and P261F, E282W mutants.

IκBα E282W HLQ

IκBα WT HLQ

IκBα P261F HLQ